

5. NONCANCER HEALTH EFFECTS OF DIESEL EXHAUST

The objective of this chapter is to evaluate the noncarcinogenic health effects of diesel exhaust. Data pertaining to exposures to whole diesel exhaust will be presented first, followed by a comparison of the effects of filtered and unfiltered exhaust. Filtered exhaust consists of the gaseous components of the exhaust without the associated particulate matter.

5.1. HEALTH EFFECTS OF WHOLE EXHAUST

5.1.1. Human Data

5.1.1.1. *Short-Term Exposures*

In a controlled human study, Rudell et al. (1990) exposed eight healthy, nonsmoking subjects for 1 h to diluted diesel exhaust. The exposure atmosphere corresponded to about 100 $\mu\text{g}/\text{m}^3$ of particles, 3.7 ppm NO, 1.6 ppm NO₂, and 0.5 mg/m³ formaldehyde (WHO, 1994). Bronchoalveolar lavage was performed prior to exposure and after exposure. Neutrophils were significantly increased after exposure, and the phagocytosis of opsonized yeast cells in vitro by alveolar macrophages was reduced after exposure. Lymphocytes were unchanged. This study provides an intriguing glimpse of the effect of low-level diesel exposure in humans, but only one exposure level was used, the number of subjects was low, and a limited range of endpoints was reported, so the data are inadequate to generalize about the human response. To date, no well-controlled chamber study has been conducted using methodologies for assessing subtle lung inflammatory reactions.

Rudell et al. (1996) exposed volunteers to diesel exhaust for 1 h in an exposure chamber. Light work on a bicycle ergometer was performed during exposure. Exposures included either unaltered diesel exhaust or exhaust with particle numbers reduced 46% by a particle trap. The engine used was a new Volvo model 1990, a six-cylinder direct-injection turbocharged diesel with an intercooler, which was run at a steady speed of 900 rpm during the exposures. Comparison of this study with others was difficult because neither exhaust dilution ratios nor particle concentrations were reported. Carbon monoxide concentrations of 27-30 ppm and NO of 2.6-2.7 ppm, however, suggested particle concentrations may have equaled several mg/m³. The most prominent symptoms during exposure were irritation of the eyes and nose and an unpleasant smell. Both airway resistance and specific airway resistance increased significantly during the exposures. Despite the 46% reduction in particle numbers by the trap, effects on symptoms and lung function were not significantly attenuated.

Kahn et al. (1988) reported the occurrence of 13 cases of acute overexposure to diesel exhaust among Utah and Colorado coal miners. Twelve miners had symptoms of mucous membrane irritation, headache, and lightheadedness. Eight individuals reported nausea; four

reported a sensation of unreality; four reported heartburn; three reported weakness, numbness, and tingling in their extremities; three reported vomiting; two reported chest tightness; and two others reported wheezing. Each miner lost time from work because of these symptoms, which resolved within 24 to 48 h. No air monitoring data were presented; poor work practices were described as the predisposing conditions for overexposure.

El Batawi and Noweir (1966) reported that among 161 workers from two garages where diesel-powered buses were serviced and repaired, 42% complained of eye irritation, 37% of headaches, 30% of dizziness, 19% of throat irritation, and 11% of cough and phlegm. Ranges of mean concentrations of diesel exhaust components in the two diesel bus garages were as follows: 0.4 to 1.4 ppm NO₂, 0.13 to 0.81 ppm SO₂, 0.6 to 44.1 ppm aldehydes, and 1.34 to 4.51 mg/m³ of particulate matter; the highest concentrations were obtained close to the exhaust systems of the buses.

Eye irritation was reported by Battigelli (1965) in six subjects after 40 s of chamber exposure to diluted diesel exhaust containing 4.2 ppm NO₂, 1 ppm SO₂, 55 ppm CO, 3.2 ppm total hydrocarbons, and 1 to 2 ppm total aldehydes; after 3 min and 20 s of exposure to diluted diesel exhaust containing 2.8 ppm NO₂, 0.5 ppm SO₂, 30 ppm CO, 2.5 ppm total hydrocarbons, and <1 to 2 ppm total aldehydes; and after 6 min of exposure to diluted diesel exhaust containing 1.3 ppm NO₂, 0.2 ppm SO₂, <20 ppm CO, <2.0 ppm total hydrocarbons, and <1.0 ppm total aldehydes. The concentration of the diesel particles was not reported.

Katz et al. (1960) described the experience of 14 chemists and their assistants monitoring the environment of a train tunnel used by diesel-powered locomotives. Although workers complained on three occasions of minor eye and throat irritation, no correlation was established with concentrations of any particular component of diesel exhaust.

5.1.1.1.1. Diesel exhaust odor. The odor of diesel exhaust is considered by most people to be objectionable; at high intensities, it may produce sufficient physiological and psychological effects to warrant concern about public health. The intensity of the odor of diesel exhaust is an exponential function of its concentration such that a tenfold change in the concentration will alter the intensity of the odor by one unit. Two human panel rating scales have been used to measure diesel exhaust odor intensity. In the first (Turk, 1967), combinations of odorous materials were selected to simulate diesel exhaust odor; a set of 12 mixtures, each having twice the concentration of that of the previous mixture, is the basis of the diesel odor intensity scale (D-scale). The second method is the TIA (total intensity of aroma) scale based on seven steps, ranging from 0 to 3, with 0 being undetectable, 1/2 very slight, and 1 slight and increasing in one-half units up to 3, strong (Odor Panel of the CRC-APRAC Program Group on Composition of Diesel Exhaust, 1979; Levins, 1981).

Surveys, utilizing volunteer panelists, have been taken to evaluate the general public's response to the odor of diesel exhaust. Hare and Springer (1971) and Hare et al. (1974) found that at a D rating of about 2 (TIA = 0.9, slight odor intensity), about 90% of the participants perceived the odor, and almost 60% found it objectionable. At a D rating of 3.2 (TIA = 1.2, slight to moderate odor intensity), about 95% perceived the odor, and 75% objected to it, and, at a D rating of 5 (TIA = 1.8, almost moderate), about 95% objected to it. Linnell and Scott (1962) evaluated the odor threshold for diesel exhaust in six subjects and found that a dilution factor of 140 to 475 was required to reduce the odor to the threshold level.

Linnell and Scott (1962) reported odor threshold measurement in six subjects and found that the dilution factor needed to reach the threshold ranged from 140 to 475 for this small sample of people. At these dilutions, the concentrations of formaldehyde ranged from 0.012 to 0.088 ppm.

5.1.1.1.2. Pulmonary and respiratory effects. Battigelli (1965) exposed 13 volunteers to three dilutions of diesel exhaust obtained from a one-cylinder, four-cycle, 7-hp diesel engine (fuel type unspecified) and found that 15-min to 1-h exposures had no significant effects on pulmonary resistance. Pulmonary resistance was measured by plethysmography utilizing the simultaneous recording of esophageal pressure and airflow determined by electrical differentiation of the volume signal from a spirometer. The units of concentration of the constituents in the three diluted exhausts were 1.3, 2.8, and 4.2 ppm NO₂; 0.2, 0.5, and 1 ppm SO₂; <20, 30, and 55 ppm CO; and <1.0, <1 to 2, and 1 to 2 ppm total aldehydes, respectively. Particle concentrations were not reported.

A number of studies have evaluated changes in pulmonary function occurring over a workshift in workers occupationally exposed to diesel exhaust (specific time period not always reported but assumed to be 8 h). In a study of coal miners, Reger et al. (1978) found that both forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) decreased by 0.05 L in 60 diesel-exposed miners, an amount not substantially different from reductions seen in non-diesel-exposed miners (0.02 and 0.04 L, respectively). Decrements in peak expiratory flow rates were similar between diesel and non-diesel exhaust-exposed miners. Miners with a history of smoking had an increased number of decrements over the shift than nonsmokers did. Although the monitoring data were not reported, the authors stated that there was no relationship between the low concentrations of measured respirable dust or NO₂ (personal samplers) when compared with shift changes for any lung function parameter measured for the diesel-exposed miners. This study is limited because results were preliminary (abstract) and there was incomplete information on the control subjects.

Ames et al. (1982) compared the pulmonary function of 60 coal miners exposed to diesel exhaust with that of a control group of 90 coal miners not exposed to diesel exhaust for evidence of acute respiratory effects associated with exposure to diesel exhaust. Changes over the workshift in FVC, FEV₁, and forced expiratory flow rate at 50% FVC (FEF₅₀) were the indices for acute respiratory effects. The environmental concentrations of the primary pollutants were 2.0 mg/m³ respirable dust (<10 µm MMAD), 0.2 ppm NO₂, 12 ppm CO, and 0.3 ppm formaldehyde. The investigators reported a statistically significant decline in FVC and FEV₁ over the workshift in both the diesel-exposed and comparisons group. Current smokers had greater decrements in FVC, FEV₁, and FEF₅₀ than exsmokers and nonsmokers. There was a marked disparity between the ages and the time spent underground for the two study groups. Diesel-exposed miners were about 15 years younger and had worked underground for 15 fewer years (4.8 versus 20.7 years) than miners not exposed to diesel exhaust. The significance of these differences between the populations studied on the results is difficult to ascertain.

Except for the expected differences related to age, 120 underground iron ore miners exposed to diesel exhaust had no workshift changes in FVC and FEV₁ when compared with 120 matched surface miners (Jørgensen and Svensson, 1970). Both groups had equal numbers (30) of smokers and nonsmokers. The frequency of bronchitis was higher among underground workers, much higher among smokers than nonsmokers, and also higher among older than younger workers. The authors reported that the underground miners had exposures of 0.5 to 1.5 ppm NO₂ and between 3 and 9 mg/m³ particulate matter with 20 to 30% of the particles <5 µm MMAD. The majority of the particles were iron ore; quartz was 6 to 7% of the fraction <5 µm MMAD.

Gamble et al. (1978) measured preshift FEV₁ and FVC in 187 salt miners and obtained peak flow forced expiratory flow rates at 25, 50, and 75% of FVC (FEF₂₅, FEF₅₀, or FEF₇₅). Postshift pulmonary function values were determined from total lung capacity and flows at preshift percentages of FVC. The miners were exposed to mean NO₂ levels of 1.5 ppm and mean respirable particulate levels of 0.7 mg/m³. No statistically significant changes were found between changes in pulmonary function and in NO₂ and respirable particles combined. Slopes of the regression of NO₂ and changes in FEV₁, FEF₂₅, FEF₅₀, and FEF₇₅ were significantly different from zero. The authors concluded that these small reductions in pulmonary function were attributable to variations in NO₂ within each of the five salt mines that contributed to the cohort.

Gamble et al. (1987a) investigated the acute effects of diesel exhaust in 232 workers in four diesel bus garages using an acute respiratory questionnaire and before and after workshift spirometry. The prevalence of burning eyes, headaches, difficult or labored breathing, nausea, and wheeze experienced at work was higher in the diesel bus garage workers than in a comparison population of lead/acid battery workers who had not previously shown a statistically significant

association of acute symptoms with acid exposure. Comparisons between the two groups were made without adjustment for age and smoking. There was no detectable association of exposure to NO₂ (0.23 ppm ± 0.24 S.D.) or inhalable (less than 10 µm MMAD) particles (0.24 mg/m³ ± 0.26 S.D.) and acute reductions in FVC, FEV₁, peak flows, FEF₅₀, and FEF₇₅. Workers who had respiratory symptoms had slightly greater but statistically insignificant reductions in FEV₁ and FEF₅₀.

Ulfvarson et al. (1987) evaluated workshift changes in the pulmonary function of 17 bus garage workers, 25 crew members of two types of car ferries, and 37 workers on roll-on/roll-off ships. The latter group was exposed primarily to diesel exhaust; the first two groups were exposed to both gasoline and diesel exhausts. The diesel-only exposures that averaged 8 h consisted of 0.13 to 1.0 mg/m³ particulate matter, 0.02 to 0.8 mg/m³ (0.016 to 0.65 ppm) NO, 0.06 to 2.3 mg/m³ (0.03 to 1.2 ppm) NO₂, 1.1 to 5.1 mg/m³ (0.96 to 4.45 ppm) CO, and up to 0.5 mg/m³ (0.4 ppm) formaldehyde. The largest decrement in pulmonary function was observed during a workshift following no exposure to diesel exhaust for 10 days. Forced vital capacity and FEV₁ were significantly reduced over the workshift (0.44 L and 0.30 L, $p < 0.01$ and $p < 0.001$, respectively). There was no difference between smokers and nonsmokers. Maximal midexpiratory flow, closing volume expressed as the percentage of expiratory vital capacity, and alveolar plateau gradient (phase 3) were not affected. Similar but less pronounced effects on FVC (-0.16 L) were found in a second, subsequent study of stevedores (n = 24) only following 5 days of no exposure to diesel truck exhaust. Pulmonary function returned to normal after 3 days without occupational exposure to diesel exhaust. No exposure-related correlation was found between the observed pulmonary effects and concentrations of NO, NO₂, CO, or formaldehyde; however, it was suggested that NO₂ adsorbed onto the diesel exhaust particles may have contributed to the overall dose of NO₂ to the lungs. In a related study, six workers (job category not defined) were placed in an exposure chamber and exposed to diluted diesel exhaust containing 0.6 mg/m³ particulate matter and 3.9 mg/m³ (2.1 ppm) NO₂. The exhaust was generated by a 6-cylinder, 2.38-L diesel engine, operated for 3 h and 40 min without interruption at constant speed, equivalent to 60 km/h, and at about one-half full engine load. No effect on pulmonary function was observed.

5.1.1.1.3. Immunological effects. The likelihood that diesel exhaust can induce reactive airway disease in humans was first reported by Wade and Newman (1993). They identified three railroad workers who developed asthma following either a single exposure or a series of short-term exposures to high concentrations of DE. Asthma diagnosis was based on symptoms, pulmonary function tests, and measurement of airway hyperreactivity to methacholine or exercise. Exposure occurred as a result of train crews riding in locomotive units trailing immediately behind the lead

engine. Although the individuals had worked for the railroad for many years and presumably had been chronically exposed to lower levels of exhaust, the symptoms developed following these subacute incidents. Unfortunately, exposure levels were not measured.

In an attempt to evaluate the potential allergenic effects of DE in humans Diaz-Sanchez and associates carried out a series of clinical investigations. In the first of these (Diaz-Sanchez et al., 1994), humans were challenged by spraying either saline or 0.30 mg DPM into their nostrils. This dose was considered equivalent to total exposure on 1-3 average days in Los Angeles, but could occur acutely in certain nonoccupational settings such as sitting at a busy bus stop or in an express tunnel. Enhanced IgE levels were noted in nasal lung lavage cells in as little as 24 h, with peak production observed 4 days after DPM challenge. The effects seemed to be somewhat isotype-specific, because in contrast to IgE results, DPM challenge had no effect on the levels of IgG, IgA, IgM, or albumin. The selective enhancement of local IgE production was demonstrated by a dramatic increase in IgE-secreting cells. Takenaka et al. (1995) reported that DPM extracts enhanced IgE production from purified human B cells. Interleukin-4 plus monoclonal antibody-stimulated IgE production was enhanced 20% to 360% by the addition of DPM extracts over a period of 10-14 days. DPM extracts themselves did not induce IgE production or synergize with interleukin-4 alone to induce IgE from purified B cells, suggesting that the extracts were enhancing ongoing IgE production rather than inducing germline transcription or isotype switching. The authors concluded that enhancement of IgE production in the human airway resulting from the organic fraction of DPM may be an important factor in the increase of allergic airway disease occurring throughout this century.

Although direct effects of DPM on B-cells have been demonstrated by in vitro studies, it was considered likely that other cells regulating the IgE response may also be affected. Cytokine production was therefore measured in nasal lung lavage cells challenged with DPM (Diaz-Sanchez et al., 1996). Before challenge, most subjects' nasal lavage cells had detectable levels of only interferon-*gamma*, IL-2, and IL-13 *mRNA*. After challenge, the cells produced readily detectable levels of *mRNA* for IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and interferon *gamma*. In addition, all levels of cytokine *mRNA* were increased. Although the cells in the nasal lavage before and after challenge do not necessarily represent the same ones either in number or type, the broad increase in cytokine production was not simply the result of an increase in T cells recovered in the lavage fluid. On the basis of these findings it was concluded that the increase in cytokine expression after exposure to DPM can be predicted to contribute to enhanced local IgE production and thus play a role in pollutant-induced airway disease.

In a continuation of the previous studies, the combined effects of intranasal challenge with DPM plus ragweed allergen were investigated. Subjects were selected on the basis of being allergic to ragweed. They were tested with doses of ragweed antigen sufficient to induce a

detectable response, 0.3 mg DPM, or ragweed allergen plus DPM combined (Diaz-Sanchez et al., 1997). As compared with challenge by ragweed alone, challenge with both DPM and ragweed allergen induced markedly higher ragweed-specific IgE, but not total IgE levels. This synergy between DPM and natural allergen exposure may be a key feature in increasing allergen-induced respiratory allergic disease.

In the available exposure studies, considerable variability is reported in diesel exhaust detection threshold. The odor scales described in some of these studies have no general use at present because they are not objectively defined; however, the studies do clearly indicate substantial interindividual variability in the ability to detect odor and the level at which it becomes objectionable. Much of what is known about the acute effects of diesel exhaust comes from case reports that lack clear measurements of exposure concentrations. The studies of pulmonary function changes in exposed humans have looked for changes occurring over a workshift or after a short-term exposure. The overall conclusion of these studies is that reversible changes in pulmonary function in humans can occur in relation to diesel exhaust exposure, although it is not possible to relate these changes to specific exposure levels. Based on the report by Wade and Newman (1993), reversible airflow obstruction and a syndrome consistent with asthma are possible following acute, high-level exposure to diesel exhaust. Recent studies by Diaz-Sanchez and co-workers have provided data indicating that DPM is a likely factor in the increasing incidence of allergic hypersensitivity. They have also shown that effects are due primarily to the organic fraction and that DPM synergizes with known allergens to increase their effectiveness.

5.1.1.2. Long-Term Exposures

Several epidemiologic studies have evaluated the effects of chronic exposure to diesel exhaust on occupationally exposed workers.

Battigelli et al. (1964) measured several indices of pulmonary function, including vital capacity, FEV₁, peak flow, nitrogen washout, and diffusion capacity in 210 locomotive repairmen exposed to diesel exhaust in three engine houses. The average exposure of these locomotive repairmen to diesel exhaust was 9.6 years. When compared with a control group matched for age, body size, "past extrapulmonary medical history" (no explanation given), and job status (154 railroad yard workers), no significant clinical differences were found in pulmonary function or in the prevalence of dyspnea, cough, or sputum between the diesel exhaust-exposed and nonexposed groups. Exposure to the diesel exhaust showed marked seasonal variations because the doors of the engine house were open in the summer and closed in the winter. For the exposed group, the maximum daily workplace concentrations of air pollutants measured were 1.8 ppm NO₂, 1.7 ppm total aldehydes, 0.15 ppm acrolein, 4.0 ppm SO₂, and 5.0 ppm total hydrocarbons. The concentration of airborne particles was not reported.

Gamble et al. (1987b) examined 283 diesel bus garage workers from four garages in two cities to determine if there was excess chronic respiratory morbidity associated with exposure to diesel exhaust. Tenure of employment was used as a surrogate of exposure; mean tenure of the study population was 9 years \pm 10 years S.D. Exposure-effect relationships within the study population showed no detectable associations of symptoms with tenure. Reductions in FVC, FEV₁, peak flow, and FEF₅₀ (but not FEF₇₅) were associated with increasing tenure. When compared with a control population (716 nonexposed blue-collar workers) and after indirect adjustment for age, race, and smoking, the exposed workers had a higher incidence of cough, phlegm, and wheezing; however, there was no correlation between symptoms and length of employment. Dyspnea showed an exposure-response trend but no apparent increase in prevalence. Mean FEV₁, FVC, FEF₅₀, and peak flow were not reduced in the total cohort compared with the reference population but were reduced in workers with 10 years or more tenure.

Purdham et al. (1987) evaluated respiratory symptoms and pulmonary function in 17 stevedores employed in car ferry operations who were exposed to both diesel and gasoline exhausts and in a control group of 11 on-site office workers. Twenty-four percent of the exposed group and 36% of the controls were smokers. If a particular symptom was considered to be influenced by smoking, smoking status was used as a covariate in the logistic regression analysis; pack-years smoked was a covariate for lung function indices. The frequency of respiratory symptoms was not significantly different between the two groups; however, baseline pulmonary function measurements were significantly different. The latter comparisons were measured by multiple regression analysis using the actual (not percentage predicted) results and correcting for age, height, and pack-years smoked. The stevedores had significantly lower FEV₁, FEV₁/FVC, FEF₅₀, and FEF₇₅ ($p < 0.021$, $p < 0.023$, $p < 0.001$, and $p < 0.008$, respectively) but not FVC. The results from the stevedores were also compared with those obtained from a study of the respiratory health status of Sydney, Nova Scotia, residents. These comparisons showed that the dock workers had higher FVC, similar FEV₁, but lower FEV₁/FVC and flow rates than the residents of Sydney. Based on these consistent findings, the authors concluded that the lower baseline function measurements in the stevedores provided evidence of an obstructive ventilatory defect but caution in interpretation was warranted because of the small sample size. There were no significant changes in lung function over the workshift, nor was there a difference between the two groups. The stevedores were exposed to significantly ($p < 0.04$) higher concentrations of particulate matter (0.06 to 1.72 mg/m³, mean 0.50 mg/m³) than the controls (0.13 to 0.58 mg/m³, mean not reported). Exposures of stevedores to SO₂, NO₂, aldehydes, and PAHs were very low; occasional CO concentrations in the 20 to 100 ppm range could be detected for periods up to 1 h in areas where blockers were chaining gasoline-powered vehicles.

Additional epidemiological studies on the health hazards posed by exposure to diesel exhaust have been conducted for mining operations. Reger et al. (1982) evaluated the respiratory health status of 823 male coal miners from six diesel-equipped mines compared with 823 matched coal miners not exposed to diesel exhaust. The average tenure of underground work for the underground miners and their controls was only about 5 years; on average, the underground workers in diesel mines spent only 3 of those 5 years underground in diesel-use mines. Underground miners exposed to diesel exhaust reported a higher incidence of symptoms of cough and phlegm but proportionally fewer symptoms of moderate to severe dyspnea than their matched counterparts. These differences in prevalence of symptoms were not statistically significant. The diesel-exposed underground miners, on the average, had lower FVC, FEV₁, FEF₅₀, FEF₇₅, and FEF₉₀ but higher peak flow and FEF₂₅ than their matched controls. These differences, however, were not statistically significant. Health indicators for surface workers and their matched controls were directionally the same as for matched underground workers. There were no consistent relationships between the findings of increased respiratory symptoms, decreased pulmonary function, smoking history, years of exposure, or monitored atmosphere pollutants (NO_x, CO, particles, and aldehydes). Mean concentrations of NO_x at the six mines ranged from 0 to 0.6 ppm for short-term area samples, 0.13 to 0.28 ppm for full-shift personal samples, and 0.03 to 0.80 for full-shift area samples. Inhalable particles (less than 10 µm MMAD) averaged 0.93 to 2.73 mg/m³ for personal samples and 0 to 16.1 mg/m³ for full shift area samples. Ames et al. (1984), using a portion of the miners studied by Reger, examined 280 diesel-exposed underground miners initially in 1977 and again in 1982. Each miner in this group had at least 1 year of underground mining work history in 1977. The control group was 838 miners with no exposure to diesel exhaust. The miners were evaluated for the prevalence of respiratory symptoms, chronic cough, phlegm, dyspnea, and changes in FVC, FEV₁, and FEF₅₀. No air monitoring data were reported; exposure to diesel exhaust gases and mine dust particles were described as very low. These authors found no decrements in pulmonary function or increased prevalence of respiratory symptoms attributable to exposure to diesel exhaust. In fact, the 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in those exposed to diesel exhaust.

Attfield (1978) studied 2,659 miners from 21 mines (8 metal, 6 potash, 5 salt, and 2 trona). Diesels were employed in only 18 of the mines, but the 3 mines not using diesels were not identified. The years of diesel usage, ranging from 8 in trona mines to 16 in potash mines, were used as a surrogate for exposure to diesel exhaust. Based on a questionnaire, an increased prevalence of persistent cough was associated with exposure to aldehydes; this finding, however, was not supported by the pulmonary function data. No adverse respiratory symptoms or pulmonary function impairments were related to CO₂, CO, NO₂, inhalable dust, or inhalable

quartz. The author failed to comment on whether the prevalence of cough was related to the high incidence (70%) of smokers in the cohort.

Questionnaire, chest radiograph, and spirometric data were collected by Attfield et al. (1982) on 630 potash miners from six potash mines. These miners were exposed for an average of 10 years (range 5 to 14 years) to 0.1 to 3.3 ppm NO₂, 0.1 to 4.0 ppm aldehyde, 5 to 9 ppm CO, and total dust concentrations of 9 to 23 mg/m³. No attempt was made to measure diesel-derived particles separately from other dusts. The ratio of total to inhalable (<10 µm MMAD) dust ranged from 2 to 11. An increased prevalence of respiratory symptoms was related solely to smoking. No association was found between symptoms and tenure of employment, dust exposure, NO₂, CO, or aldehydes. A higher prevalence of symptoms of cough and phlegm was found, but no differences in pulmonary function (FVC and FEV₁) were found in these diesel-exposed potash miners when compared with the predicted values derived from a logistics model based on blue-collar workers working in nondusty jobs.

Gamble et al. (1983) investigated respiratory morbidity in 259 miners from five salt mines in terms of increased respiratory symptoms, radiographic findings, and reduced pulmonary function associated with exposure to NO₂, inhalable particles (<10 µm MMAD), or years worked underground. Two of the mines used diesel extensively; no diesels were used in one salt mine. Diesels were introduced into each mine in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared with the salt miner cohort. After adjustment for age and smoking, the salt miners showed no increased prevalence of cough, phlegm, dyspnea, or airway obstruction (FEV₁/FVC) compared with aboveground coal miners, potash miners, or blue-collar workers. The underground coal miners consistently had an elevated level of symptoms. Forced expiratory volume at 1 s, FVC, FEF₅₀, and FEF₇₅ were uniformly lower for salt miners in relation to all the comparison populations. There was, however, no association between changes in pulmonary function and years worked, estimated cumulative inhalable particles, or estimated NO₂ exposure. The highest average exposure to particulate matter was 1.4 mg/m³ (particle size not reported, measurement includes NaCl). Mean NO₂ exposure was 1.3 ppm, with a range of 0.17 ppm to 2.5 ppm. In a continuation of these studies, Gamble and Jones (1983) grouped the salt miners into low-, intermediate-, and high-exposure categories based on tenure in jobs with diesel exhaust exposure. Average concentrations of inhalable particles and NO₂ were 0.40, 0.60, and 0.82 mg/m³ and 0.64, 1.77, and 2.21 ppm for the three diesel exposure categories, respectively. A statistically significant concentration-response association was found between the prevalence of phlegm in the salt miners and exposure to diesel exhaust ($p < 0.0001$) and a similar, but nonsignificant, trend for cough and dyspnea. Changes in pulmonary function showed no association with diesel tenure. In a comparison with the control group of nonexposed, blue-collar workers, adjusted for age and smoking, the overall prevalence of cough and phlegm (but not

dyspnea) was elevated in the diesel-exposed workers. Forced expiratory volumes at 1 s and FVC were within 4% of expected, which was considered to be within the normal range of variation for a nonexposed population.

In a preliminary study of three subcohorts from bus company personnel (clerks [lowest exposure], bus drivers [intermediate exposure], and bus garage workers [highest exposure]) representing different levels of exposure to diesel exhaust, Edling and Axelson (1984) found a fourfold higher risk ratio for cardiovascular mortality in bus garage workers, even after adjusting for smoking history and allowing for at least 10 years of exposure and 15 years or more of induction-latency. Carbon monoxide was hypothesized as the etiologic agent for the increased cardiovascular disease but was not measured. However, in a more comprehensive epidemiological study, Edling et al. (1987) evaluated mortality data covering a 32-year period for a cohort of 694 bus garage employees and found no significant differences between the observed and expected number of deaths from cardiovascular disease. Information on exposure components and their concentrations was not reported.

The absence of reported noncancerous human health effects, other than infrequently occurring effects related to respiratory symptoms and pulmonary function changes, is notable. Unlike studies in laboratory animals to be described later in this chapter, studies of the impact of diesel exhaust on the defense mechanisms of the human lung have not been performed. No direct evidence is available in humans regarding doses of diesel exhaust, gas phase, particulate phase, or total exhaust that lead to impaired particle clearance or enhanced susceptibility to infection. A summary of epidemiology studies is presented in Table 5-1.

To date, no large-scale epidemiological study has looked for effects of chronic exposure to diesel exhaust on pulmonary function. In the long-term longitudinal and cross-sectional

Table 5-1. Human studies of exposure to diesel exhaust

Study	Description	Findings
Acute exposures		
Kahn et al. (1988)	13 cases of acute exposure, Utah and Colorado coal miners.	Acute reversible sensory irritation, headache, nervous system effects, bronchoconstriction were reported at unknown exposures.
El Batawi and Noweir (1966)	161 workers, two diesel bus garages.	Eye irritation (42%), headache (37%), dizziness (30%), throat irritation (19%), and cough and phlegm (11%) were reported in this order of incidence by workers exposed in the service and repair of diesel-powered buses.
Battigelli (1965)	Six subjects, eye exposure chamber, three dilutions.	Time to onset was inversely related and severity of eye irritation was associated with the level of exposure to diesel exhaust.
Katz et al. (1960)	14 persons monitoring diesel exhaust in a train tunnel.	Three occasions of minor eye and throat irritation; no correlation established with concentrations of diesel exhaust components.
Hare and Springer (1971) Hare et al. (1974)	Volunteer panelists who evaluated general public's response to odor of diesel exhaust.	Slight odor intensity, 90% perceived, 60% objected; slight to moderate odor intensity, 95% perceived, 75% objected; moderate odor intensity, 100% perceived, almost 95% objected.
Linnell and Scott (1962)	Odor panel under highly controlled conditions determined odor threshold for diesel exhaust.	In six panelists, the volume of air required to dilute raw diesel exhaust to an odor threshold ranged from a factor of 140 to 475.
Ruddell et al. (1988)	Volunteers exposed to about 100 µg/m ³ DPM.	Neutrophils were increased and phagocytosis of opsonized yeast cells was reduced after exposure.
Rudell et al. (1996)	Volunteers exposed to diesel exhaust for one hour while doing light work. Exposure concentrations uncertain.	Unpleasant smell along with irritation of eyes and nose reported. Airway resistance increased. Reduction of particle concentration by trapping did not affect results.
Battigelli (1965)	13 volunteers exposed to three dilutions of diesel exhaust for 15 min to 1 h.	No significant effects on pulmonary resistance were observed as measured by plethysmography.
Wade and Newman (1993)	Three railroad workers acutely exposed to diesel exhaust.	The workers developed symptoms of asthma.
Diaz-Sanchez et al. (1994)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	Enhancement of IgE production reported due to a dramatic increase in IgE-secreting cells.
Takenaka et al. (1995)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	DPM extracts enhanced interleukin-4 plus monoclonal antibody-stimulated IgE production as much as 360%, suggesting an enhancement of ongoing IgE production rather than inducing germline transcription or isotype switching.
Diaz-Sanchez et al. (1996)	Volunteers challenged by a nasal spray of 0.30 mg DPM.	A broad increase in cytokine expression predicted to contribute to enhanced local IgE production.

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Diaz-Sanchez et al. (1997)	Ragweed-sensitive volunteers challenged by a nasal spray of 0.30 mg DPM alone or in combination with ragweed allergen.	Ragweed allergen plus DPM-stimulated ragweed-specific IgE to a much greater degree than ragweed alone, suggesting DPM may be a key feature in stimulating allergen-induced respiratory allergic disease.
Studies of cross-shift changes		
Reger et al. (1978)	Five or more VC maneuvers by each of 60 coal miners exposed to diesel exhaust at the beginning and end of a workshift.	FEV ₁ , FVC, and PEFR were similar between diesel and non-diesel-exposed miners. Smokers had an increased number of decrements over shift than nonsmokers.
Ames et al. (1982)	Pulmonary function of 60 diesel-exposed compared with 90 non-diesel-exposed coal miners over workshift.	Significant workshift decrements occurred in miners in both groups who smoked; no significant differences in ventilatory function changes between miners exposed to diesel exhaust and those not exposed.
Jørgensen and Svensson (1970)	240 iron ore miners matched for diesel exposure, smoking, and age were given bronchitis questionnaires and spirometry pre- and postworkshift.	Among underground (surrogate for diesel exposure) miners, smokers, and older age groups, frequency of bronchitis was higher. Pulmonary function was similar between groups and subgroups except for differences accountable to age.
Gamble et al. (1978)	200 salt miners performed before and after workshift spirometry. Personal environmental NO ₂ and inhalable particle samples were collected.	Smokers had greater but not significant reductions in spirometry than ex- or nonsmokers. NO ₂ but not particulate levels significantly decreased FEV ₁ , FEF ₂₅ , FEF ₅₀ , and FEF ₇₅ over the workshift.
Gamble et al. (1987a)	232 workers in four diesel bus garages administered acute respiratory questionnaire and before and after workshift spirometry. Compared to lead/acid battery workers previously found to be unaffected by their exposures.	Prevalence of burning eyes, headache, difficult or labored breathing, nausea, and wheeze were higher in diesel bus workers than in comparison population.
Ulfvarson et al. (1987)	Workshift changes in pulmonary function were evaluated in crews of roll-on/ roll-off ships and car ferries and bus garage staff. Pulmonary function was evaluated in six volunteers exposed to diluted diesel exhaust, 2.1 ppm NO ₂ , and 0.6 mg/m ³ particulate matter.	Pulmonary function was affected during a workshift exposure to diesel exhaust, but it normalized after a few days with no exposure. Decrement was greater with increasing intervals between exposures. No effect on pulmonary function was observed in the experimental exposure study.

Cross-sectional and longitudinal studies

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Battigelli et al. (1964)	210 locomotive repairmen exposed to diesel exhaust for an average of 9.6 years in railroad engine houses were compared with 154 railroad yard workers of comparable job status but no exposure to diesel exhaust.	No significant differences in VC, FEV ₁ , peak flow, nitrogen washout, or diffusion capacity or in the prevalence of dyspnea, cough, or sputum were found between the diesel exhaust-exposed and nonexposed groups.
Gamble et al. (1987b)	283 male diesel bus garage workers from four garages in two cities were examined for impaired pulmonary function (FVC, FEV ₁ , and flow rates). Study population with a mean tenure of 9 ± 10 years S.D. was compared to a nonexposed blue-collar population.	Analyses within the study population showed no association of respiratory symptoms with tenure. Reduced FEV ₁ and FEF ₅₀ (but not FEF ₇₅) were associated with increasing tenure. The study population had a higher incidence of cough, phlegm, and wheezing unrelated to tenure. Pulmonary function was not affected in the total cohort of diesel-exposed but was reduced with 10 or more years of tenure.
Purdham et al. (1987)	Respiratory symptoms and pulmonary function were evaluated in 17 stevedores exposed to both diesel and gasoline exhausts in car ferry operations; control group was 11 on-site office workers.	No differences between the two groups for respiratory symptoms. Stevedores had lower baseline lung function consistent with an obstructive ventilatory defect compared with controls and those of Sydney, Nova Scotia, residents. Caution in interpretation is warranted due to small sample size. No significant changes in lung function over workshift or difference between two groups.
Reger et al. (1982)	Differences in respiratory symptoms and pulmonary function were assessed in 823 coal miners from six diesel-equipped mines compared to 823 matched coal miners not exposed to diesel exhaust.	Underground miners in diesel-use mines reported more symptoms of cough and phlegm and had lower pulmonary function. Similar trends were noted for surface workers at diesel-use mines. Pattern was consistent with small airway disease but factors other than exposure to diesel exhaust thought to be responsible.
Ames et al. (1984)	Changes in respiratory symptoms and function were measured during a 5-year period in 280 diesel-exposed and 838 nonexposed U.S. underground coal miners.	No decrements in pulmonary function or increased prevalence of respiratory symptoms were found attributable to diesel exhaust. In fact, 5-year incidences of cough, phlegm, and dyspnea were greater in miners without exposure to diesel exhaust than in miners exposed to diesel exhaust.
Attfield (1978)	Respiratory symptoms and function were assessed in 2,659 miners from 21 underground metal mines (1,709 miners) and nonmetal mines (950 miners). Years of diesel usage in the mines were surrogate for exposure to diesel exhaust.	Questionnaire found an association between an increased prevalence of cough and aldehyde exposure; this finding was not substantiated by spirometry data. No adverse symptoms or pulmonary function decrements were related to exposure to NO ₂ , CO, CO ₂ , dust, or quartz.

Table 5-1. Human studies of exposure to diesel exhaust (continued)

Study	Description	Findings
Attfield et al. (1982)	Respiratory symptoms and function were assessed in 630 potash miners from six potash mines using a questionnaire, chest radiographs, and spirometry. A thorough assessment of the environment of each mine was made concurrently.	No obvious association indicative of diesel exposure was found between health indices, dust exposure, and pollutants. Higher prevalences of cough and phlegm but no differences in FVC and FEV ₁ were found in these diesel-exposed potash workers when compared with predicted values from a logistic model based on blue-collar staff working in nondusty jobs.
Gamble et al. (1983)	Respiratory morbidity was assessed in 259 miners in five salt mines by respiratory symptoms, radiographic findings, and spirometry. Two mines used diesels extensively, two had limited use, one used no diesels in 1956, 1957, 1963, or 1963 through 1967. Several working populations were compared with the salt mine cohort.	After adjustment for age and smoking, salt miners showed no symptoms, increased prevalence of cough, phlegm, dyspnea, or air obstruction (FEV ₁ /FVC) compared with aboveground coal miners, potash workers, or blue-collar workers. FEV ₁ , FVC, FEF ₅₀ , and FEF ₇₅ were uniformly lower for salt miners in comparison with all the comparison populations. No changes in pulmonary function were associated with years of exposure or cumulative exposure to inhalable particles or NO ₂ .
Gamble and Jones (1983)	Same as above. Salt miners were grouped into low-, intermediate-, and high-exposure categories based on tenure in jobs with diesel exposure.	A statistically significant dose-related association of phlegm and diesel exposure was noted. Changes in pulmonary function showed no association with diesel tenure. Age- and smoking-adjusted rates of cough, phlegm, and dyspnea were 145, 169, and 93% of an external comparison population. Predicted pulmonary function indices showed small but significant reductions; there was no dose-response relationship.
Edling and Axelson (1984)	Pilot study of 129 bus company employees classified into three diesel-exhaust exposure categories: clerks (0), bus drivers (1), and bus garage workers.	The most heavily exposed group (bus garage workers) had a fourfold increase in risk of dying from cardiovascular disease, even after correction for smoking and allowing for 10 years of exposure and 15 years or more of induction latency time.
Edling et al. (1987)	Cohort of 694 male bus garage employees followed from 1951 through 1983 was evaluated for mortality from cardiovascular disease. Subcohorts categorized by levels of exposure were clerks (0), bus drivers (1), and bus garage employees (2).	No increased mortality from cardiovascular disease was found among the members of these five bus companies when compared with the general population or grouped as subcohorts with different levels of exposure.

studies, a relationship was generally observed between work in a job with diesel exposure and respiratory symptoms (such as cough and phlegm), but there was no consistent effect on pulmonary function. The interpretation of these results is hampered by lack of measured diesel exhaust exposure levels and the short duration of exposure in these cohorts. Only active workers were included in these studies. It is possible that the relationship between work in a job with diesel exposure and respiratory symptoms was due to short-term exposure.

5.1.2. Laboratory Animal Studies

Because of the large number of statistical comparisons made in the laboratory animal studies and to permit uniform, objective evaluations within and among studies, data will be reported as significantly different (i.e., $p < 0.05$) unless otherwise specified. The exposure regimens used and the resultant exposure conditions employed in the laboratory animal inhalation studies are summarized in Appendix A. Other than the pulmonary function studies performed by Wiester et al. (1980) on guinea pigs during their exposure in inhalation chambers, the pulmonary function studies performed by other investigators, although sometimes unreported, were interpreted as being conducted on the following day or thereafter and not immediately following exposure.

5.1.2.1. Acute Exposures

The acute toxicity of undiluted diesel exhaust to rabbits, guinea pigs, and mice was assessed by Pattle et al. (1957). Four engine operating conditions were used, and 4 rabbits, 10 guinea pigs, and 40 mice were tested under each exposure condition for 5 h (no controls were used). Mortality was assessed up to 7 days after exposure. With the engine operating under light load, the exhaust was highly irritating but not lethal to the test species, and only mild tracheal and lung damage was observed in the exposed animals. The exhaust contained 74 mg/m³ particulate matter (particle size not reported), 560 ppm CO, 23 ppm NO₂, and 16 ppm aldehydes. Exhaust containing 5 mg/m³ particulate matter, 380 ppm CO, 43 ppm NO₂, and 6.4 ppm aldehydes resulted in low mortality rates (mostly below 10%) and moderate lung damage. Exhaust containing 122 mg/m³ particulate matter, 418 ppm CO, 51 ppm NO₂, and 6.0 ppm aldehydes produced high mortality rates (mostly above 50%) and severe lung damage. Exhaust containing 1,070 mg/m³ particulate matter, 1,700 ppm CO, 12 ppm NO₂, and 154 ppm aldehydes resulted in 100% mortality in all three species. High CO levels, which resulted in a carboxyhemoglobin value of 60% in mice and 50% in rabbits and guinea pigs, were considered to be the main cause of death in the latter case. High NO₂ levels were considered to be the main cause of lung damage and mortality seen in the other three tests. Aldehydes and NO₂ were considered to be the main irritants in the light load test.

Kobayashi et al. (1995) administered 1, 10, or 20 mg/kg DPM for 30 min to the nasal mucosa of guinea pigs. The administration increased nasal airway resistance, augmented increased airway resistance and nasal secretion induced by a histamine aerosol, increased vascular permeability in dorsal skin, and augmented vacular permeability induced by histamine. The increases in nasal airway resistance and secretion are considered typical responses of nasal mucosa against allergic stimulation. Similar results were reported for guinea pigs exposed via inhalation for 3 h to diesel exhaust diluted to particle concentrations of either 1 or 3.2 mg/m³ (Kobayashi et al., 1997). These studies show that short-term exposure to diesel exhaust potently induces nasal mucosal hyperresponsiveness in guinea pigs.

The effects of diesel exhaust particles (DPM) and their components (extracted particles and particle extracts) on the release of proinflammatory cytokines, interleukin-1, and tumor necrosis factor- α (TNF- α) by alveolar macrophages (AM) were investigated by Yang et al. (1997). Rat AM were incubated with 0, 5, 10, 20, 50, or 100 μ g/10⁶ AM/mL of DPM, methanol-extracted DPM, or equivalent concentrations of DPM at 37 °C for 24 h. At high concentrations, both DPM and DPM extracts were shown to increase IL-1-like activity secreted by AM, whereas extracted particles had no effect. Neither particles, particle extracts, or extracted particles stimulated secretion of TNF α . DPM inhibited lipid polysaccharide (LPS)-stimulated production of IL-1 and TNF- α . In contrast, interferon- γ stimulated production of IL-1 and TNF- α .

Results of this study indicate that the organic fraction of exhaust particles is responsible for the effects noted. Stimulation of IL-1 but not TNF- α suggests that IL-1, but not TNF- α , may play an important role in the development of DPM-induced inflammatory and immune responses. The cellular mechanism involved in inhibiting increased release of IL-1 and TNF- α by LPS is unknown, but may be a contributing factor to the decreased AM phagocytic activity and increased susceptibility to pulmonary infection after prolonged exposure to DPM.

Takano et al. (1997) conducted a study designed to evaluate the effects of DPM on the manifestations of allergic asthma in mice, with emphasis on antigen-induced airway inflammation, the local expression of interleukin-5 (IL-5), macrophage colony stimulating factor (GM-CSF), IL-2 and interferon (IFN)- γ , and the production of antigen-specific IgE and IgG. Male ICR mice were intratracheally instilled with ovalbumin (OVA), DPM, and DPM+OVA. DPM was obtained from a 4JB1-type, light-duty 2.74 L, four-cylinder Izuzu diesel engine operated at a steady speed of 1,500 rpm under a load of 10 torque (kg/m). The OVA-group mice were instilled with 1 μ g OVA at 3 and 6 weeks. The mice receiving DPM alone were instilled with 100 μ g DPM weekly for 6 weeks. The OVA + DPM group received the combined treatment in the same protocol as the OVA and the DPM groups, respectively. Additional groups were exposed for 9 weeks. DPM aggravated ovalbumin-induced airway inflammation, characterized by infiltration of eosinophils and lymphocytes and an increase in goblet cells in the bronchial epithelium. DPM in

combination with antigen markedly increased IL-5 protein levels in lung tissue and bronchoalveolar lavage supernatants compared with either antigen or DPM alone. The combination of DPM and antigen induced significant increases in local expression of IL-4, GM-CSF, and IL-2, whereas expression of IFN-gamma was not affected. In addition, DPM exhibited adjuvant activity for the antigen-specific production of IgG and IgE. These results provide experimental evidence that DPM can enhance the manifestations of allergic asthma and suggest that DPM is implicated in the increasing prevalence of allergic asthma in recent years.

5.1.2.2. *Short-Term and Subchronic Exposures*

A number of inhalation studies have employed a regimen of 20 h/day, 7 days/week for varying exposure periods up to 20 weeks to differing concentrations of airborne particulate matter, vapor, and gas concentrations of diluted diesel exhaust. Exposure regimens and characterization of gas-phase components for these studies are summarized in Table 5-2. Pepelko et al. (1980a) evaluated the pulmonary function of cats exposed under these conditions for 28 days to 6.4 mg/m³ particulate matter. The only significant functional change observed was a decrease in maximum expiratory flow rate at 10% vital capacity. The excised lungs of the exposed cats appeared charcoal gray, with focal black spots visible on the pleural surface. Pathologic changes included a predominantly peribronchial localization of black-pigmented macrophages within the alveoli characteristic of focal pneumonitis or alveolitis.

The effects of a short-term diesel exhaust exposure on arterial blood gases, pH, blood buffering, body weight changes, lung volumes, and deflation pressure-volume (PV) curves of young adult rats were evaluated by Pepelko (1982a). Exposures were 20 h/day, 7 days/week for 8 days to a concentration of 6.4 mg/m³ particulate matter in the nonirradiated exhaust (RE) and 6.75 mg/m³ in the irradiated exhaust (IE). In spite of the irradiation, levels of gaseous compounds were not substantially different between the two groups (Table 5-2). Body weight gains were significantly reduced in the reexposed rats and to an even greater degree in rats exposed to IE. Arterial blood gases and standard bicarbonate were unaffected, but arterial blood pH was significantly reduced in rats exposed to IE. Residual volume and wet lung weight were not affected by either exposure, but vital capacity and total lung capacity were increased significantly following exposure to RE. The shape of the deflation PV curves were nearly identical for the control, RE and IE groups.

Table 5-2. Short-term effects of diesel exhaust on laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 10-13 weeks	1.5 0.19 µm MMD	2,100 to 2,730	6.9	0.49	—	Increase in lung wt; increase in thickness of alveolar walls; minimal species difference	Kaplan et al. (1982)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 19 weeks	0.21 1.0 4.4	140 665 2,926	— — —	— — —	— — —	No effects on lung function in rats (not done in mice); increase in PMNs and proteases and AM aggregation in both species	Mauderly et al. (1981)
Cat, Inbred, M	20 h/day 7 days/week 4 weeks	6.4	3,584	14.6	2.1	2.1	Few effects on lung function; focal pneumonitis or alveolitis	Pepelko et al. (1980a)
Rat, Sprague-Dawley, M	20 h/day 7 days/week 4 weeks	6.4 6.8 ^a	3,584 3,808	16.9 16.1 ^a	2.49 2.76 ^a (<0.01 ppm O ₃) ^a	2.10 1.86 ^a	Decreased body wt; arterial blood pH reduced; vital capacity, total lung capacities increased	Pepelko (1982a)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 4 weeks	6.8 ^a	3,808	16.7	2.9 (<0.01 ppm O ₃) ^a	1.9	Exposure started when animals were 4 days old; increase in pulmonary flow; bradycardia	Wiester et al. (1980)
Rat, F344, M	20 h/day 5.5 days/week 4 weeks	6.0 6.8 µm MMD	2,640	—	—	—	Macrophage aggregation; increase in PMNs; Type II cell proliferation; thickened alveolar walls	White and Garg (1981)
Guinea Pig, Hartley, M	30 min	1-2 mg DPM Intranasally	—	—	—	—	Augmented increases in nasal airway resistance and vascular permeability induced by a histamine aerosol	Kobayashi et al. (1995)
Guinea Pig, Hartley, M	3 h	1 3.2	0.5 1.6	5.9 12.9	1.4 4.4	0.13 0.34	Similar results to those reported in the previous study using intranasal challenge	Kobayashi et al. (1997)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 8 weeks	6.3	7,056	17.4	2.3	2.1	Increase in relative lung wt. AM aggregation; hypertrophy of goblet cells; focal hyperplasia of alveolar epithelium	Wiester et al. (1980)

Table 5-2. Short-term effects of diesel exhaust on laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mouse ICR, M	6 weeks	100 µg DPM intranasally	—	—	—	—	DPM aggravated ovalbumin-induced airway inflammation and provided evidence that DPM can enhance manifestations of allergic asthma	Takano et al. (1997)
Rat, Sprague-Dawley, M	24 h	5-100 µg/10 ⁶ AM/ml of DPM	—	—	—	—	Unchanged, but not organic-free DPM enhanced production of proinflammatory cytokines	Yang et al. (1997)

^aIrradiated exhaust.
PMN = Polymorphonuclear leukocyte.
AM = Alveolar macrophage.

In related studies, Wiester et al. (1980) evaluated pulmonary function in 4-day-old guinea pigs exposed for 20 h/day, 7 days/week for 28 days to IE having a concentration of 6.3 mg/m³ particulate matter. When housed in the exposure chamber, pulmonary flow resistance increased 35%, and a small but significant sinus bradycardia occurred as compared with controls housed and measured in control air chambers ($p < 0.002$). Respiratory rate, tidal volume, minute volume, and dynamic compliance were unaffected as were lead-I electrocardiograms.

A separate group of adult guinea pigs was necropsied after 56 days of exposure to IE, to diluted RE, or to clean air (Wiester et al., 1980). Exposure resulted in a significant increase in the ratio of lung weight to body weight (0.68% for controls, 0.78% for IE, and 0.82% for RE). Heart/body weight ratios were not affected by exposure. Microscopically, there was a marked accumulation of black pigment-laden alveolar macrophages (AM) throughout the lung with a slight to moderate accumulation in bronchial and carinal lymph nodes. Hypertrophy of goblet cells in the tracheobronchial tree was frequently observed, and focal hyperplasia of alveolar lining cells was occasionally observed. No evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.

White and Garg (1981) studied pathologic alterations in the lungs of rats (16 exposed and 8 controls) after exposure to diesel exhaust containing 6 mg/m³ particulate matter. Two rats from the exposed group and one rat from the control group (filtered room air) were sacrificed after each exposure interval of 6 h and 1, 3, 7, 14, 28, 42, and 63 days; daily exposures were for 20 h and were 5.5 days/week. Evidence of AM recruitment and phagocytosis of diesel particles was found at the 6-h sacrifice; after 24 h of exposure there was a focal, scattered increase in the number of Type II cells. After 4 weeks of exposure, there were morphologic changes in size, content, and shape of AM, septal thickening adjacent to clusters of AMs, and an appearance of inflammatory cells, primarily within the septa. At 9 weeks of exposure, focal aggregations of particle-laden macrophages developed near the terminal bronchi, along with an influx of accumulation of black pigment-laden alveolar macrophages (AM) throughout the lung with a slight to moderate accumulation in bronchial and carinal lymph nodes. Hypertrophy of goblet cells in the tracheobronchial tree was frequently observed, and focal hyperplasia of alveolar lining cells was occasionally observed. No evidence of squamous metaplasia of the tracheobronchial tree, emphysema, peribronchitis, or peribronchiolitis was noted.

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number of Type II cells. After 4 weeks of exposure, there were morphologic changes in size, content, and shape of AM, septal thickening adjacent to clusters of AMs, and an appearance of inflammatory cells, primarily within the septa. At 9 weeks of exposure, focal aggregations of particle-laden macrophages developed near the terminal bronchi, along with an influx of polymorphonuclear leukocytes (PMNs), Type II cell proliferation, and thickening of the alveolar walls. The affected alveoli occurred in clusters that, for the most part, were located near the terminal bronchioles, but occasionally were focally located in the lung parenchyma.

Mauderly et al. (1981) exposed rats and mice by inhalation to diluted diesel exhaust for 545 h over a 19-week period on a regimen of 7 h/day, 5 days/week at concentrations of 0, 0.21, 1.02, or 4.38 mg/m³ particulate matter. Indices of health effects were minimal following 19 weeks of exposure. There were no significant exposure-related differences in mortality or body weights of the rats or mice. There also were no significant differences in respiratory function (breathing patterns, dynamic lung mechanics, lung volumes, quasi-static PV relationships, forced expirograms, and CO-diffusing capacity) in rats; pulmonary function was not measured in mice. No effect on tracheal mucociliary or deep lung clearances were observed in the exposed groups. Rats, but not mice, had elevated immune responses in lung-associated lymph nodes at the two higher exposure levels. Inflammation in the lungs of rats exposed to 4.38 mg/m³ particulate matter was indicated by increases in PMNs and lung tissue proteases. Histopathologic findings included AMs that contained diesel particles, an increase in Type II cells, and the presence of particles in the interstitium and tracheobronchial lymph nodes.

Kaplan et al. (1982) evaluated the effects of subchronic exposure to diesel exhaust on rats, hamsters, and mice. The exhaust was diluted to a concentration of 1.5 mg/m³ particulate matter; exposures were 20 h/day, 7 days/week. Hamsters were exposed for 86 days, rats and mice for 90 days. There were no significant differences in mortality or growth rates between exposed and control animals. Lung weight relative to body weight of 15 rats exposed for 90 days was significantly higher than the mean for a control group (15 rats). Histological examination of tissues of all three species indicated particle accumulation in the lungs and mediastinal lymph nodes. Associated with the larger accumulations, there was a minimal increase in the thickness of the alveolar walls, but the vast majority of the particles elicited no response. After 6 mo of recovery, considerable clearance of the diesel particles from the lungs occurred in all three species, as evaluated by gross pathology and histopathology. However, no quantitative estimate of clearance was provided.

Toxic effects in animals from acute exposure to diesel exhaust appear to be primarily attributable to the gaseous components (i.e., mortality from CO intoxication and lung injury caused by cellular damage resulting from NO₂ exposure). The results from short-term exposures indicate that rats experience no to minimal lung function impairment even at diesel exhaust levels

sufficiently high to cause histological and cytological changes in the lung. In subchronic studies of durations of 4 weeks or more, frank adverse health effects are not readily apparent and when found are mild and result from exposure to concentrations of about 6 mg/m³ particulate matter and durations of exposures of 20 h/day. There is ample evidence that subchronic exposure to lower levels of diesel exhaust affects the lung, as indicated by accumulation of particles, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and thickening of alveolar walls adjacent to AM aggregates. Little evidence exists, however, that subchronic exposure to diesel exhaust impairs lung function. Recent studies have implicated the organic fraction of DPM in the induction of respiratory allergic disease.

5.1.2.3. Chronic Exposures

5.1.2.3.1. Effects on growth and longevity. Changes in growth, body weight, absolute or relative organ weights, and longevity can be measurable indicators of chronic toxic effects. Such effects have been observed in some but not all of the long-term studies conducted on laboratory animals exposed to diesel exhaust. There was limited evidence for an effect on survival in the published chronic animal studies; deaths occurred intermittently early in one study in female rats exposed to 3.7 mg/m³ particulate matter; however, the death rate began to decrease after 15 mo, and the survival rate after 30 mo was slightly higher than that of the control group (Research Committee for HERP Studies, 1988). Studies of the effects of chronic exposure to diesel exhaust on survival and body weight or growth are detailed in Table 5-3.

Increased lung weights and lung-to-body weight ratios have been reported in rats, mice, and hamsters. These data are summarized in Table 5-4. In rats exposed for up to 36 weeks to 0.25 or 1.5 mg/m³ particulate matter, lung wet weights (normalized to body weight) were significantly higher in the 1.5 mg/m³ exposure group than control values after 12 weeks of exposure (Misiorowski et al., 1980). Rats and Syrian hamsters were exposed for 2 years (five 16-h periods per week) to diesel exhaust diluted to achieve concentrations of 0.7, 2.2, and 6.6 mg/m³ particulate matter (Brightwell et al., 1986). At necropsy, a significant increase in lung weight was seen in both rats and hamsters exposed to diesel exhaust compared with controls. This finding was more pronounced in the rats in which the increase was progressive with both duration of exposure and particulate matter level. The increase was greatest at 30 mo (after the end of a 6-month observation period in the high-concentration male group where the lung weight was 2.7 times the control and at 24 mo in the high-concentration female group [3.9 times

Table 5-3. Effects of chronic exposures to diesel exhaust on survival and growth of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M, F; Monkey, cynomolgus, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MMD	7,280	11.5	1.5	0.8	No effects on growth or survival	Lewis et al. (1989)
Rat, F344, M; Guinea Pig, Hartley, M	20 h/day 5 days/week 106 weeks	0.25 0.75 1.5 0.19 µm MMD	2,650 7,950 15,900	2.7 ^a 4.4 ^a 7.1 ^a	0.1 ^b 0.27 ^b 0.5 ^b	— — —	Reduced body weight in rats at 1.5 mg/m ³	Schreck et al. (1981)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	— —	No effect on growth	Vinegar et al. (1981a,b)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8.3 0.71 µm MMD	21,663	50.0	4.0–6.0	—	No effect on growth or mortality rates	Karagianes et al. (1981)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.0 0.25 µm MMD	1,592 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	No effect on growth or mortality rates	Mauderly et al. (1984, 1987a)
Rat, Wistar, F; Mouse, MMRI, F	19 h/day 5 days/week 104 weeks	4.24 0.35 µm MMD	41,891	12.5	1.5	1.1	Reduced body wts; increased mortality in mice	Heinrich et al. (1986a)
Rat, F344 M, F	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Growth reduced at 2.2 and 6.6 mg/m ³	Brightwell et al. (1986)
Rat ^c F344/Jcl.	16 h/day 6 days/week 130 weeks	0.11 ^d 0.41 ^d 1.08 ^d 2.31 ^d 3.72 ^e 0.2–0.3 µm MMD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Concentration-dependent decrease in body weight; earlier deaths in females exposed to 3.72 mg/m ³ , stabilized by 15 mo	Research Committee for HERP Studies (1988)
Rat, Wistar, F; Mouse, NMRI, F (7 mg/m ³ only)	18 h/day 5 days/week 24 mo	0.84 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Reduced body weight in rats at 2.5 and 6.98 mg/m ³ and no effect in mice	Heinrich et al. (1995)

Table 5-3. Effects of chronic exposures to diesel exhaust on survival and growth of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mice, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	Reduced body weight in NMRI mice but not in C57BL/6N mice	
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Reduced survival in 6.33 mg/m ³ after 300 days. Body weight significantly lower at 6.33 mg/m ³	Nikula et al. (1995)

^aEstimated from graphically depicted mass concentration data.

^bEstimated from graphically presented mass concentration data for NO₂(assuming 90% NO and 10% NO₂).

^cData for tests with light-duty engine; similar results with heavy-duty engine.

^dLight-duty engine.

^eHeavy-duty engine.

Table 5-4. Effects of chronic exposures to diesel exhaust on organ weights and organ-to-body-weight ratios

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 12-13 weeks	1.5 0.19 µm MMD	2,520-2,730	—	—	—	No effect on liver, kidney, spleen, or heart weights	Kaplan et al. (1982)
Rat, F344, M, F	7 h/day 5 days/week 52 weeks	2.0 0.23–0.36 µm MMD	3,640	12.7	1.6	0.83	No effects on weights of lungs, liver, heart, spleen, kidneys, and testes	Green et al. (1983)
Rat, F344, M	20 h/day 5.5 days/week 36 weeks	0.25 1.5 0.19 µm MMD	990 5,940	— —	— —	—	Increase in relative lung weight at 1.5 mg/m ³ only initially seen at 12 weeks	Misiorowski et al. (1980)
Rat, F344, F	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MMD	7,280	11.5	1.5	0.81	No effects on heart weights	Vallyathan et al. (1986)
Rat, F344; M Guinea Pig, Hartley, M	20 h/day 5.5 days/week 78 weeks	0.25 0.75 1.5 0.19 µm MMD	2,145 6,435 12,870	— — —	— — —	—	No effects on heart mass	Penney et al. (1981)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	—	Increase in lung weight and lung/body weight ratio	Vinegar et al. (1981a,b)
Rat, Wistar, F; Hamster, Syrian, M, F Mouse, NMRI, F	19 h/day 5 days/week 120-140 weeks	4.24 0.35 µm MMD	48,336-56,392	12.5	1.5	1.1	Increase in rat, mouse, and hamster lung weight and dry weights	Heinrich et al. (1986a,b) Stöber (1986)
Rat, F344, M, F; Hamster, Syrian, M, F	16 h/day 5 days/week 104 weeks	0.7 ^a 2.2 ^b 6.6	5,824 18,304 54,912	— — 32.0	— — —	—	Increase in lung weight concentration related in rats; heart weight/body weight ratio greater at 6.6 mg/m ³	Brightwell et al. (1986)
Cat inbred, M	8 h/day 7 days/week 124 weeks	6.0 ^a 12.0 ^b	41,664 83,328	20.2 33.2	2.7 4.4	2.7 5.0	Decrease in lung and kidney weights	Pepelko et al. (1980b, 1981) Moorman et al. (1985)
Mouse, NMRI, F (7 mg/m ³ only)	18 h/day 5 days/week 24 mo	0.84 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Increased rat and mouse lung weight at 7 mg/m ³ from 6 mo and at 2.5 mg/m ³ at 22 and 24 mo	Heinrich et al. (1995)

Table 5-4. Effects of chronic exposures to diesel exhaust on organ weights and organ-to-body-weight ratios (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	Increased lung weight	
Rats, F344, M	16 h/day 5 days/week 23 mo	2.44 6.33	19,520 50,640	— —	— —	— —	Increase in lung weight was significant at 2 and 6 mg/m ³	Nikula et al. (1995)
Rat		0.8 2.5 6.98					Increased lung weight in rats and mice at 3.5 and 7 mg/m ³	Henderson et al. (1988)
Mouse		6.98 4.5						

^a1 to 61 weeks of exposure.
^b62 to 124 weeks of exposure.

control]). Heinrich et al. (1986a,b; see also Stöber, 1986) found a significant increase in wet and dry weights of the lungs of rats and mice exposed at 4.24 mg/m³ particulate matter for 1 year in comparison with controls. After 2 years, the difference was a factor of 2 (mice) or 3 (rats). After the same exposure periods, the hamsters showed increases of 50 to 75%, respectively. Exposure to equivalent filtered diesel exhaust caused no significant effects in any of the species. Vinegar et al. (1980, 1981a,b) exposed hamsters to two levels of diesel exhaust with resultant concentrations of about 6 and 12 mg/m³ particulate matter for 8 h/day, 7 days/week for 6 mo. Both exposures significantly increased lung weight and lung weight to body weight ratios. The difference between lung weights of exposed and control hamsters exposed to 12 mg/m³ particulate matter was approximately twice that of those exposed to 6 mg/m³.

Heinrich et al. (1995) reported that rats exposed to 2.5 and 7 mg/m³ for 18 h/day, 5 days/week for 24 mo showed significantly lower body weights than control starting at day 200 in the high-concentration group and at day 440 in the low-concentration group. Body weight in the low-concentration group was unaffected, as was mortality in any group. Lung weight was increased in the 7 mg/m³ group starting at 3 mo and persisting throughout the study while the 2.5 mg/m³ group showed increased lung weight only at 22 and 24 mo of exposure. Mice (NMRI strain) exposed to 7 mg/m³ in this study for 13.5 mo had no increase in mortality and minimal, insignificant decreases in body weight. Lung weights were dramatically affected, with increases progressing throughout the study from 1.5-fold at 3 mo to 3-fold at 12 mo. Mice (NMRI and C57BL/6N strains) were also exposed to 4.5 mg/m³ for 23 mo. In NMRI mice, the body weights were reported to be significantly lower than controls, but the magnitude of the change is not reported so biological significance cannot be assessed. Mortality was slightly increased, but statistical significance is not reported. The C57BL/6N mice showed minimal effects on body weight and mortality, which were not reported to be statistically significant. Lung weights were dramatically affected in both strains.

Nikula et al. (1995) exposed male and female F344 rats to diesel particle concentrations of 2.4 and 6.3 mg/m³ for 16 h/day, 5 days/week, for 23 mo in a study designed to compare the effects of diesel with those of carbon black. Significantly reduced survival was observed in males exposed to 6.3 mg/m³ but not in females or at the lower concentration. Body weights were decreased by exposure to 6.3 mg/m³ diesel exhaust in both male and female rats throughout the exposure period. Significant increases in lung weight were first seen at 6 mo in the high-exposure group and at 12 to 18 mo in the low-exposure group.

No evidence was found in the published literature that chronic exposure to diesel exhaust affected the weight of body organs other than the lung and heart (e.g., liver, kidney, spleen, or testes) (Table 5-4). Morphometric analysis of hearts from rats and guinea pigs exposed to 0.25, 0.75, or 1.5 mg/m³ particulate matter 20 h/day, 5.5 days/week for 78 weeks revealed no

significant alteration in mass at any exposure level or duration of exposure (Penney et al., 1981). The analysis included relative wet weights of the right ventricle, left ventricle, combined atria, and ratio of right to left ventricle. Vallyathan et al. (1986) found no significant differences in heart weights and the ratio of heart weight to body weight between rats exposed to 2 mg/m³ particulate matter for 7 h/day, 5 days/week for 24 mo and their respective clean air chamber controls. No significant differences were found in the lungs, heart, liver, spleen, kidney, and testes of rats exposed for 52 weeks, 7 h/day, 5 days/week to diluted diesel exhaust containing 2 mg/m³ particulate matter compared with their respective controls (Green et al., 1983).

5.1.2.3.2. *Effects on pulmonary function.* The effect of long-term exposure to diesel exhaust on pulmonary function has been evaluated in laboratory studies of rats, hamsters, cats, and monkeys. These studies are summarized in Table 5-5, along with more details on the exposure characteristics, in general order of increasing dose ($C \times T$) of the diesel exhaust particulate matter. The text will be presented using the same approach.

Lewis et al. (1989) evaluated 10 control and 10 diesel-exposed rats (2 mg/m³ particulate matter, 7 h/day, 5 days/week for 52 or 104 weeks) for responses in functional residual capacity and airway resistance and conductance. At the 104-week evaluation, the rats were also examined for maximum flow volume impairments. No evidence of an impairment of pulmonary function as a result of the exposure to diesel exhaust was found in rats. Lewis et al. (1989) exposed male cynomolgus monkeys to diesel exhaust for 7 h/day, 5 days/week, for 24 mo. Groups of 15 monkeys were exposed to air, diesel exhaust (2 mg/m³), coal dust, or combined coal dust and diesel exhaust. Pulmonary function was evaluated prior to exposure and at 6-month intervals during the 2-year exposure, including compliance and resistance, static and dynamic lung volumes, distribution of ventilation, diffusing capacity, and maximum ventilatory performance. There were no effects on lung volumes, diffusing capacity, or ventilation distribution, so there was no evidence of restrictive disease. There was, however, evidence of obstructive airway disease as measured by low maximal flows in diesel-exposed monkeys. At 18 mo of exposure, forced expiratory flow at 25% of vital capacity and forced expiratory flow normalized to FVC were increased. The measurement of forced expiratory flow at 40% of total lung capacity was significantly increased at 12, 18, and 24 mo of exposure. The finding of an obstructive effect in monkeys contrasts with the finding of restrictive type effects in other laboratory animal species (Vinegar et al., 1980, 1981a; Mauderly et al., 1988; Pepelko et al., 1980b, 1981) and suggests a possible difference in effect between primate and small animal respiratory tracts. In these

Table 5-5. Effects of diesel exhaust on pulmonary function of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MMD	7,280	11.5	1.5	0.8	No effect on pulmonary function	Lewis et al. (1989)
Monkey, M, Cynomolgus	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MMD	7,280	11.5	1.5	0.8	Decreased expiratory flow; no effect on vital or diffusing capacities	Lewis et al. (1989)
Rat, F344, M	20 h/day 5.5 days/week 87 weeks	1.5 0.19 µm MMD	14,355	7.0	0.5	—	Increased functional residual capacity, expiratory volume, and flow	Gross (1981)
Rat, Wistar, F	7-8 h/day 5 days/week 104 weeks	3.9 0.1 µm MMD	14,196–16,224	18.5	1.2	3.1	No effect on minute volume, compliance, or resistance	Heinrich et al. (1982)
Hamster, Chinese, M	8 h/day 7 days/week 26 weeks	6.0 12.0	8,736 17,472	— —	— —	— —	Decrease in vital capacity, residual volume, and diffusing capacity; increase in static deflation lung volume	Vinegar et al. (1980, 1981a,b)
Rat, F344, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.0 0.23–0.26 µm MMD	1,593 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Diffusing capacity, lung compliance reduced at 3.5 and 7 mg/m ³	Mauderly et al. (1988) McClellan et al. (1986)
Hamster, Syrian, M, F	19 h/day 5 days/week 120 weeks	4.24 0.35 µm MMD	48,336	12.5	1.5	1.1	Significant increase in airway resistance	Heinrich et al. (1986a)
Rat, F344, M, F; Hamster Syrian, M, F	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — —	— — —	— — —	Large number of pulmonary function changes consistent with obstructive and restrictive airway diseases at 6.6 mg/m ³ (no specific data provided)	Brightwell et al. (1986)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4.24 0.35 µm MMD	56,392	12.5	1.5	1.1	Decrease in dynamic lung compliance; increase in airway resistance	Heinrich et al. (1986a)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0 ^a 12.0 ^b	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	Decrease in vital capacity, total lung capacity, and diffusing capacity after 2 years; no effect on expiratory flow	Pepelko et al. (1980b, 1981) Moorman et al. (1985)

^a1 to 61 weeks exposure.^b62 to 124 weeks of exposure.

monkeys there were no specific histopathological effects reported (see next section) although particle aggregates were reported in the distal airways, suggesting more small airway deposition.

Gross (1981) exposed rats for 20 h/day, 5.5 days/week for 87 weeks to diesel exhaust containing 1.5 mg/m³ particulate matter. When the data were normalized (e.g., indices expressed in units of airflow or volume for each animal by its own forced expiratory volume), there were no apparent functionally significant changes occurring in the lungs at 38 weeks of exposure that might be attributable to the inhalation of diesel exhaust. After 87 weeks of exposure, functional residual capacity (FRC) and its component volumes (expiratory reserve [ER] and residual volume [RV]), maximum expiratory flow (MEF) at 40% FVC, MEF at 20% FVC, and FEV_{0.1} were significantly greater in the diesel-exposed rats. An observed increase in airflow at the end of the forced expiratory maneuver when a decreased airflow would be expected from the increased FRC, ER, and RV data (the typical scenario of human pulmonary disease) showed these data to be inconsistent with known clinically significant health effects. Furthermore, although the lung volume changes in the diesel-exposed rats could have been indicative of emphysema or chronic obstructive lung disease, this interpretation was contradicted by the airflow data, which suggest simultaneous lowering of the resistance of the distal airways.

Heinrich et al. (1982) evaluated the pulmonary function of rats exposed to a concentration of 3.9 mg/m³ particulate matter for 7 to 8 h/day, 5 days/week for 2 years. When compared with a control group, no significant changes in respiratory rate, minute volume, compliance, or resistance occurred in the exposed group (number of rats per group was not stated).

Hamsters (eight or nine per group) were exposed 8 h/day, 7 days/week, for 6 mo to concentrations of either about 6 mg/m³ or about 12 mg/m³ particulate matter (Vinegar et al., 1980, 1981a,b). Vital capacity, vital capacity/lung weight ratio, residual lung volume by water displacement, and CO₂ diffusing capacity decreased significantly in hamsters exposed to 6 mg/m³ particulate matter. Static deflation volume-pressure curves showed depressed deflation volumes for diesel-exposed hamsters when volumes were corrected for body weight and even greater depressed volumes when volumes were corrected for lung weight. However, when volumes were expressed as percentage of vital capacity, the diesel-exposed hamsters had higher lung volumes at 0 and 5 cm H₂O. In the absence of confirmatory histopathology, the authors tentatively concluded that these elevated lung volumes and the significantly reduced diffusing capacity in the same hamsters were indicative of possible emphysematous changes in the lung. Similar lung function changes were reported in hamsters exposed at 12 mg/m³ particulate matter, but detailed information was not reported. It was stated, however, that the decrease in vital capacity was 176% greater in the second experiment than in the first.

Mauderly et al. (1988; see also McClellan et al., 1986) examined the impairment of respiratory function in rats exposed for 7 h/day, 5 days/week, for 24 mo to diluted diesel exhaust

with 0.35, 3.5, or 7.1 mg/m³ particulate matter. After 12 mo of exposure to the highest concentration of diesel exhaust, the exposed rats (n = 22) had lower total lung capacity (TLC), dynamic lung compliance (C_{dyn}), FVC, and CO diffusing capacity than controls (n = 23). After 24 mo of exposure to 7 mg/m³ particulate matter, mean TLC, C_{dyn}, quasi-static chord compliance, and CO diffusing capacity were significantly lower than control values. Nitrogen washout and percentage of FVC expired in 0.1 s were significantly greater than control values. There was no evidence of airflow obstruction. The functional alterations were attributed to focal fibrotic and emphysematous lesions and thickened alveolar membranes observed by histological examination. Similar functional alterations and histopathologic lesions were observed in the rats exposed to 3.5 mg/m³ particulate matter, but such changes usually occurred later in the exposure period and were generally less pronounced. There were no significant decrements in pulmonary function for the 0.35 mg/m³ group at any time during the study nor were there reported histopathologic changes in this group.

Additional studies were conducted by Heinrich et al. (1986a,b; see also Stöber, 1986) on the effects of long-term exposure to diesel exhaust on the pulmonary function of hamsters and rats. The exhaust was diluted to achieve a concentration of 4.24 mg/m³ particulate matter; exposures were for 19 h/day, 5 days/week for a maximum of 120 weeks (hamsters) or 140 weeks (rats). After 1 year of exposure to the diesel exhaust, the hamsters exhibited a significant increase in airway resistance and a nonsignificant reduction in lung compliance. For the same time period, rats showed increased lung weights, a significant decrease in C_{dyn}, and a significant increase in airway resistance. These indices did not change during the second year of exposure.

Syrian hamsters and rats were exposed to 0.7, 2.2, or 6.6 mg/m³ particulate matter for five 16-h periods per week for 2 years (Brightwell et al., 1986). There were no treatment-related changes in pulmonary function in the hamster. Rats exposed to the highest concentration of diesel exhaust exhibited changes in pulmonary function (data not presented) that were reported to be consistent with a concentration-related obstructive and restrictive disease.

Pepelko et al. (1980b; 1981; see also Pepelko, 1982b) and Moorman et al. (1985) measured the lung function of adult cats chronically exposed to diesel exhaust. The cats were exposed for 8 h/day and 7 days/week for 124 weeks. Exposures were at 6 mg/m³ for the first 61 weeks and 12 mg/m³ from weeks 62 to 124. No definitive pattern of pulmonary function changes was observed following 61 weeks of exposure; however, a classic pattern of restrictive lung disease was found at 124 weeks. The significantly reduced lung volumes (TLC, FVC, FRC, and inspiratory capacity [IC]) and the significantly lower single-breath diffusing capacity, coupled with normal values for dynamic ventilatory function (mechanics of breathing), indicate the presence of a lesion that restricts inspiration but does not cause airway obstruction or loss of elasticity. This

pulmonary physiological syndrome is consistent with an interstitial fibrotic response that was later verified by histopathology (Plopper et al., 1983).

Pulmonary function impairment has been reported in rats, hamsters, cats, and monkeys chronically exposed to diesel exhaust. In all species but the monkey, the pulmonary function testing results have been consistent with restrictive lung disease. The monkeys demonstrated evidence of small airway obstructive responses. The disparity between the findings in monkeys and those in rats, hamsters, and cats could be in part the result of increased particle retention in the smaller species resulting from (1) exposure to diesel exhaust that has higher airborne concentrations of gases, vapors, and particles and/or (2) longer duration of exposure. The nature of the pulmonary impairment is also dependent on the site of deposition and routes of clearance, which are determined by the anatomy and physiology of the test laboratory species and the exposure regimen. The data on pulmonary function effects raise the possibility that diesel exhaust produces small airway disease in primates compared with primarily alveolar effects in small animals and that similar changes might be expected in humans and monkeys. Unfortunately, the available data in primates are too limited to draw clear conclusions.

5.1.2.3.3. Lung morphology, biochemistry, and lung lavage analysis. Several studies have examined the morphological, histological, and histochemical changes occurring in the lungs of laboratory animals chronically exposed to diesel exhaust. The histopathological effects of diesel exposure in the lungs of laboratory animals are summarized in Table 5-6, ranked in order of C × T. Table 5-6 also contains an expanded description of exposures.

Kaplan et al. (1982) performed macroscopic and microscopic examinations of the lungs of rats, mice, and hamsters exposed for 20 h/day, 7 days/week for 3 mo to diesel exhaust containing 1.5 mg/m³ particulate matter. Gross examination revealed diffuse and focal deposition of the diesel particles that produced a grayish overall appearance of the lungs with scattered, denser black areas. There was clearance of particles via the lymphatics to regional lymph nodes. Microscopic examination revealed no anatomic changes in the upper respiratory tract; the mucociliary border was normal in appearance. Most of the particles were in macrophages, but some were free as small aggregates on alveolar and bronchiolar surfaces. The particle-laden macrophages were often in masses near the entrances of the lymphatic drainage and respiratory ducts. Associated with these masses was a minimal increase in the thickness of the alveolar walls; however, the vast majority of the particles elicited no response. After 6 mo

Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals (continued)**Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals**

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M; Mouse, A/J, M; Hamster, Syrian, M	20 h/day 7 days/week 12-13 weeks	1.5 0.19 µm MDD	2,520-2,730	—	—	—	Inflammatory changes; increase in lung weight; increase in thickness of alveolar walls	Kaplan et al. (1982)
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MDD	7,280	11.5	1.5	0.8	AM aggregation; no fibrosis, inflammation, or emphysema	Lewis et al. (1989)
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MDD	3,640	11.5	1.5	0.8	Multifocal histiocytosis; inflammatory changes; Type II cell proliferation; fibrosis	Bhatnagar et al. (1980) Pepelko (1982a)
Rat, Sprague-Dawley, M; Mouse, A/HEJ, M	8 h/day 7 days/week 39 weeks	6.0	13,104	—	—	—	Increase in lung protein content and collagen synthesis but a decrease in overall lung protein synthesis in both species; prolylhydroxylase activity increased in rats in utero	Bhatnagar et al. (1980) Pepelko (1982a)
Hamster, Chinese, M	8 h/day 5 days/week 26 weeks	6.0 12.0	6,240 12,480	— —	— —	—	Inflammatory changes; AM accumulation; thickened alveolar lining; Type II cell hyperplasia; edema; increase in collagen	Pepelko (1982b)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 120 weeks	3.9 0.1 µm MDD	16,380-18,720	18.5	1.2	3.1	Inflammatory changes, 60% adenomatous cell proliferation	Heinrich et al. (1982)
Rat, Wistar, M	6 h/day 5 days/week 87 weeks	8.3 0.71 µm MDD	21,663	50.0	4.0-6.0	—	Inflammatory changes; AM aggregation; alveolar cell hypertrophy; interstitial fibrosis, emphysema (diagnostic methodology not described)	Karagianes et al. (1981)
Rat, F344, F	8 h/day 7 days/week 104 weeks	4.9	28,538	7.0	1.8	13.1	Type II cell proliferation; inflammatory changes; bronchial hyperplasia; fibrosis	Iwai et al. (1986)
Rat, F344, M, F; Mouse, CD-1, M, F	7 h/day 5 days/week 130 weeks	0.35 3.5 7.1 0.23 µm MDD	1,592 15,925 31,850	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Alveolar and bronchiolar epithelial metaplasia in rats at 3.5 and 7.0 mg/m ³ ; fibrosis at 7.0 mg/m ³ in rats and mice; inflammatory changes	Mauderly et al. (1987a) Henderson et al. (1988)
Rat, Wistar, F; Mouse, NMRI, F (7 mg/m ³ only)	18 h/day 5 days/week 24 mo	0.8 2.5 6.98	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.2 3.8	0.3 1.1 3.4	Bronchioalveolar hyperplasia, interstitial fibrosis in all groups. Severity and incidence increase with exposure concentration	Heinrich et al. (1995)

Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mouse, NMRI, F; C57BL/6N, F	18 h/day 5 days/week 13.5 mo (NMRI) 24 mo (C57BL/N)	6.98	35,500 - NMRI 38,300 - C57	14.2	2.3	2.8	No increase in tumors. Noncancer effects not discussed	
Mouse		4.5					No increase in tumors Noncancer effects not discussed	
Rat, M, F, F344/Jcl.	16 h/day 6 days/week 130 weeks	0.11 ^a 0.41 ^a 1.08 ^a 2.31 ^a 3.72 ^b	1,373 5,117 13,478 28,829 46,336	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	Inflammatory changes; Type II cell hyperplasia and lung tumors seen at >0.4 mg/m ³ ; shortening and loss of cilia in trachea and bronchi	Research Committee for HERP Studies (1988)
Hamster, Syrian, M, F	19 h/day 5 days/week 120 weeks	4.24	48,336	12.5	1.5	1.1	Inflammatory changes; thickened alveolar septa; bronchioloalveolar hyperplasia; emphysema (diagnostic methodology not described)	Heinrich et al. (1986a)
Mouse, NMRI, F	19 h/day 5 days/week 120 weeks	4.24	48,336	12.5	1.5	1.1	Inflammatory changes; bronchioloalveolar hyperplasia; alveolar lipoproteinosis; fibrosis	Heinrich et al. (1986a)
Rat, Wistar, F	19 h/day 5 days/week 140 weeks	4.24	56,392	12.5	1.5	1.1	Thickened alveolar septa; AM aggregation; inflammatory changes; hyperplasia; lung tumors	Heinrich et al. (1986a)
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 104 weeks	0.25 0.75 1.5 6.0	2,860 8,580 17,160 68,640	— — — —	— — — —	— — — —	Minimal response at 0.25 and ultrastructural changes at 0.75 mg/m ³ ; thickened alveolar membranes; cell proliferation; fibrosis at 6.0 mg/m ³ ; increase in PMN at 0.75 mg/m ³ and 1.5 mg/m ³	Barnhart et al. (1981, 1982) Vostal et al. (1981)

Table 5-6. Histopathological effects of diesel exhaust in the lungs of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Cat, inbred, M	8 h/day	6.0 ^c	41,664	20.2	2.7	2.1	Inflammatory changes; AM aggregation; bronchiolar epithelial metaplasia; Type II cell hyperplasia; peribronchiolar fibrosis	Plopper et al. (1983)
	7 days/week 124 weeks	12.0 ^d	83,328	33.2	4.4	5.0		Hyde et al. (1985)
Rat, F344, M	16 h/day	2.44	19,520	—	—	—	AM hyperplasia, epithelial hyperplasia, inflammation, septal fibrosis, bronchoalveolar metaplasia	Nikula et al. (1995)
	5 days/week 23 mo	6.33	50,640	—	—	—		

^aLight-duty engine.^bHeavy-duty engine.^c1 to 61 weeks exposure.^d62 to 124 weeks of exposure.

AM = Alveolar macrophage.

PMN = Polymorphonuclear leukocyte.

of recovery, the lungs of all three species contained considerably less pigment, as assessed by gross pathological and histopathological examinations.

Lewis et al. (1989; see also Green et al., 1983) performed serial histological examinations of rat lung tissue exposed to diesel exhaust containing 2 mg/m³ particulate matter for 7 h/day, 7 days/week for 2 years. Accumulations of black-pigmented AMs were seen in the alveolar ducts adjacent to terminal bronchioles as early as 3 mo of exposure, and particles were seen within the interstitium of the alveolar ducts. These macular lesions increased in size up to 12 mo of exposure. Collagen or reticulum fibers were seen only rarely in association with deposited particles; the vast majority of lesions showed no evidence of fibrosis. There was no evidence of focal emphysema with the macules. Multifocal histiocytosis (24% of exposed rats) was observed only after 24 mo of exposure. These lesions were most commonly observed subpleurally and were composed of collections of degenerating macrophages and amorphous granular material within alveoli, together with fibrosis and chronic inflammatory cells in the interstitium. Epithelial lining cells adjacent to collections of pigmented macrophages showed a marked Type II cell hyperplasia; degenerative changes were not observed in Type I cells. Histological examination of lung tissue from monkeys exposed for 24 mo in the same regimen as used for rats revealed aggregates of black particles, principally in the distal airways of the lung. Particles were present within the cytoplasm of macrophages in the alveolar spaces as well as the interstitium. Fibrosis, focal emphysema, or inflammation was not observed. No specific histopathological lesions were reported for the monkey.

Nikula et al. (1997) reevaluated the lung tissue from this study. They concluded that there were no significant differences in the amount of retained particulate matter between monkeys and rats exposed under the same conditions. The rats, however, retained a greater portion of the particulate matter in lumens of the alveolar ducts and alveoli than did the monkeys. Conversely, monkeys retained a greater portion of the particulate material in the interstitium than did rats. Aggregations of particle-laden macrophages in the alveoli were rare, and there were few signs of particle-associated inflammation in the monkeys. Minimal histopathologic lesions were detected in the interstitium. Although the lungs of the monkeys showed a marginal and significantly lesser inflammatory response than rats exposed to the same exposure regime, the results should be interpreted with caution because 2 years is near the normal lifetime for rats, but less than 10% of the normal lifespan of *Cynomolgus* monkeys.

Histopathological effects of diesel exhaust on the lungs of rats have been investigated by the Health Effects Research Program on Diesel Exhaust (HERP) in Japan. Both light-duty (LD) and heavy-duty (HD) diesel engines were used. The exhaust was diluted to achieve nominal concentrations of 0.1 (LD only), 0.4 (LD and HD), 1 (LD and HD), 2 (LD and HD), and 4 (HD only) mg/m³ particulate matter. Rats were exposed for 16 h/day, 6 days/week for 30 mo. No

histopathological changes were observed in the lungs of rats exposed to 0.4 mg/m³ particulate matter or less. At concentrations above 0.4 mg/m³ particulate matter, severe morphological changes were observed. These changes consisted of shortened and absent cilia in the tracheal and bronchial epithelium, marked hyperplasia of the bronchiolar epithelium, and swelling of the Type II cellular epithelium. These lesions appeared to increase in severity with increases in exhaust concentration and duration of exposure. There was no difference in the degree of changes in pulmonary pathology at the same level of concentrations between the LD and the HD series.

Histological examination of the respiratory tract of hamsters revealed significantly higher numbers of hamsters exhibiting definite proliferative changes in the lungs in the group exposed to diesel exhaust than were observed in the group exposed to particle-free diesel exhaust or clean air (Heinrich et al., 1982). Sixty percent of these changes were described as adenomatous proliferations. Exposures were for 7 to 8 h/day, 5 days/week for 104 weeks to diesel exhaust diluted to achieve a concentration of 3.9 mg/m³ particulate matter.

Heinrich et al. (1995) reported increased incidence and severity of bronchioloalveolar hyperplasia in rats exposed to 0.8, 2.5, and 7 mg/m³. The lesion in the lowest concentration group was described as very slight to moderate. Slight to moderate interstitial fibrosis also increased in incidence and severity in all exposed groups, but incidences were not reported. This chronic study also exposed NMRI mice to 7 mg/m³ for 13.5 mo and both NMRI and C56BL/6N mice to 4.5 mg/m³ for 24 mo. Noncancer histological endpoints are not discussed in any detail in the report, which is focused on the carcinogenicity on diesel as compared with titanium dioxide and carbon black.

Iwai et al. (1986) performed serial histopathology on the lungs of rats at 1, 3, 6, 12, and 24 mo of exposure to diesel exhaust. Exposures were for 8 h/day, 7 days/week for 24 mo; the exposure atmosphere contained 4.9 mg/m³ particulate matter. At 1 and 3 mo of exposure, there were minimal histological changes in the lungs of the exposed rats. After 6 mo of exposure, there were particle-laden macrophages distributed irregularly throughout the lung and a proliferation of Type II cells with adenomatous metaplasia in areas where the macrophages had accumulated. After 1 year of exposure, foci of heterotrophic hyperplasia of ciliated or nonciliated bronchiolar epithelium on the adjacent alveolar walls were more common, the quantity of deposited particulate matter increased, and the number of degenerative AMs and proliferative lesions of Type II or bronchiolar epithelial cells increased. After 2 years of exposure, there was a fibrous thickening of the alveolar walls, mast cell infiltration with epithelial hyperplasia in areas where the macrophages had accumulated, and neoplasms.

Heinrich et al. (1986a; see also Stöber, 1986) performed histopathologic examinations of the respiratory tract of hamsters, mice, and rats exposed to diesel exhaust that had 4 mg/m³ particulate matter. Exposures were for 19 h/day, 5 days/week; the maximum exposure period

was 120 weeks for hamsters and mice and 140 weeks for rats. Histological examination revealed different levels of response among the three species. In hamsters, the exhaust produced thickened alveolar septa, bronchioloalveolar hyperplasia, and what were termed emphysematous lesions (diagnostic methodology not described). In mice, bronchioloalveolar hyperplasia occurred in 64% of the mice exposed to the exhaust and in 5% of the controls. Multifocal alveolar lipoproteinosis occurred in 71% and multifocal interstitial fibrosis occurred in 43% of the mice exposed to exhaust but in only 4% of the controls. In exposed rats, there were severe inflammatory changes in the lungs, as well as thickened septa, foci of macrophages, and hyperplastic and metaplastic lesions.

Nikula et al. (1995) reported in detail the nonneoplastic effects in male and female F344 rats exposed to 2.4 or 6.3 mg/m³ of diesel exhaust particles. At 3 mo in the low-concentration group, enlarged particle-containing macrophages were found with minimal aggregation. With higher concentration and longer duration of exposure, the number and size of macrophages and aggregates increased. Alveolar epithelial hyperplasia was found starting at 3 mo and in all rats at 6 mo. These lesions progressed to chronic active inflammation, alveolar proteinosis, and septal fibrosis at 12 mo. Other lesions observed late in the study included bronchiolar-alveolar metaplasia, squamous metaplasia, and squamous cysts. This study reports in detail the progression of lesions in diesel exhaust exposure and finds relatively little difference between the lesions caused by diesel exhaust exposure and exposure to similar levels of carbon black particles.

The effects of diesel exhaust on the lungs of 18-week-old rats exposed to 8.3 ± 2.0 mg/m³ particulate matter were investigated by Karagianes et al. (1981). Exposures were for 6 h/day, 5 days/week, for 4, 8, 16, or 20 mo. Histological examinations of lung tissue noted focal aggregation of particle-laden AMs, alveolar histiocytosis, interstitial fibrosis, and alveolar emphysema (diagnostic methodology not described). Lesion severity was related to length of exposure. No significant differences were noted in lesion severity among the diesel exhaust, the diesel exhaust plus coal dust (5.8 ± 3.5 mg/m³), or the high-concentration (14.9 ± 6.2 mg/m³) coal dust exposure groups following 20 mo of exposure.

Histological changes in the lungs of guinea pigs exposed to diluted diesel exhaust containing either 0.25, 0.75, 1.5, or 6.0 mg/m³ particulate matter were reported by Barnhart et al. (1981, 1982). Exposures at 0.75 and 1.5 mg/m³ for 2 weeks to 6 mo resulted in an uptake of exhaust particles by three alveolar cell types (AMs, Type I cells, and interstitial macrophages) and also by granulocytic leukocytes (eosinophils). The alveolar-capillary membrane increased in thickness as a result of an increase in the absolute tissue volume of interstitium and Type II cells. In a continuation of these studies, guinea pigs were exposed to diesel exhaust (up to 6.0 mg/m³ particulate matter) for 2 years (Barnhart et al., 1982). A minimal tissue response occurred at the concentration of 0.25 mg/m³. After 9 mo of exposure, there was a significant increase, about

30%, in Type I and II cells, endothelial cells, and interstitial cells over concurrent age-matched controls; by 24 mo only macrophages and Type II cells were significantly increased. As in the earlier study, ultrastructural evaluation showed that Type I cells, AMs, and eosinophils phagocytized the diesel particles. Exposure to 0.75 mg/m³ for 6 mo resulted in fibrosis in regions of macrophage clusters and in focal Type II cell proliferation. No additional information was provided regarding the fibrotic changes with increasing concentration or duration of exposure. With increasing concentration/duration of diesel exhaust exposure, Type II cell clusters occurred in some alveoli. Intraalveolar debris was particularly prominent after exposures at 1.5 and 6.0 mg/m³ and consisted of secretory products from Type II cells.

In studies conducted on hamsters, Pepelko (1982b) found that the lungs of hamsters exposed for 8 h/day, 7 days/week for 6 mo to 6 or 12 mg/m³ particulate matter were characterized by large numbers of black AM in the alveolar spaces, thickening of the alveolar epithelium, hyperplasia of Type II cells, and edema.

Lungs from rats and mice exposed to 0.35, 3.5, or 7.1 mg/m³ (0.23 to 0.26 μ m mass median diameter [MMD]) for 7 h/day and 5 days/week showed pathologic lesions (Mauderly et al., 1987a; Henderson et al., 1988). After 1 year of exposure at 7.1 mg/m³, the lungs of the rats exhibited focal areas of fibrosis; fibrosis increased with increasing duration of exposure and was observable in the 3.5-mg/m³ group of rats at 18 mo. The severity of inflammatory responses and fibrosis was directly related to the exposure level. In the 0.35 mg/m³ group of rats, there was no inflammation or fibrosis. Although the mouse lungs contained high lung burdens of diesel particles per gram of lung weight at each equivalent exposure concentration, there was substantially less inflammatory reaction and fibrosis than was the case in rats. Fibrosis was observed only in the lungs of mice exposed at 7 mg/m³ and consisted of fine fibrillar thickening of occasional alveolar septa.

Histological examinations were performed on the lungs of cats initially exposed to 6 mg/m³ particulate matter for 61 weeks and subsequently increased to 12 mg/m³ for Weeks 62 to 124 of exposure. Plopper et al. (1983; see also Hyde et al., 1985) concluded from the results of this study that exposure to diesel exhaust produced changes in both epithelial and interstitial tissue compartments and that the focus of these lesions in the peripheral lung was the centriacinar region where the alveolar ducts join the terminal conducting airways. This conclusion was based on the following evidence. The epithelium of the terminal and respiratory bronchioles in exposed cats consisted of three cell types (ciliated, basal, and Clara cells) compared with only one type (Clara cells) in the controls. The proximal acinar region showed evidence of peribronchial fibrosis and bronchiolar epithelial metaplasia. Type II cell hyperplasia was present in the proximal interalveolar septa. The more distal alveolar ducts and the majority of the rest of the parenchyma were unchanged from controls. Peribronchial fibrosis was greater at the end of 6 mo in clean air

following exposure, whereas the bronchiolar epithelial metaplasia was most severe at the end of exposure. Following an additional 6 mo in clean air, the bronchiolar epithelium more closely resembled the control epithelial cell population.

Wallace et al. (1987) used transmission electron microscopy (TEM) to determine the effect of diesel exhaust on the intravascular and interstitial cellular populations of the lungs of exposed rats and guinea pigs. Exposed animals and matched controls were exposed to 0.25, 0.75, 1.5, or 6.0 mg/m³ particulate matter for 2, 6, or 10 weeks or 18 mo. The results inferred the following: (1) exposure to 6.0 mg/m³ for 2 weeks was insufficient to elicit any cellular response, (2) both species demonstrated an adaptive multicellular response to diesel exhaust, (3) increased numbers of fibroblasts were found in the interstitium from week 6 of exposure through month 18, and (4) there was no significant difference in either cell type or number in alveolar capillaries, but there was a significant increase at 18 mo in the mononuclear population in the interstitium of both species.

Additional means for assessing the adverse effects of diesel exhaust on the lung are to examine biochemical and cytological changes in bronchoalveolar lavage fluid (BALF) and in lung tissue. Fedan et al. (1985) performed studies to determine whether chronic exposure of rats affected the pharmacologic characteristics of the rat's airway smooth muscle. Concentration-response relationships for tension changes induced with acetylcholine, 5-hydroxytryptamine, potassium chloride, and isoproterenol were assessed in vitro on isolated preparations of airway smooth muscle (trachealis). Chronic exposure to diesel exhaust significantly increased the maximal contractile responses to acetylcholine compared with control values; exposure did not alter the sensitivity (EC₅₀ values) of the muscles to the agonists. Exposures were to diesel exhaust containing 2 mg/m³ particulate matter for 7 h/day, 5 days/week for 2 years.

Biochemical studies of BALF obtained from hamsters and rats revealed that exposures to diesel exhaust caused significant increases in lactic dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase (G6P-DH), total protein, collagen, and protease (pH 5.1) after approximately 1 year and 2 years of exposure (Heinrich et al., 1986a). These responses were generally much greater in rats than in hamsters. Exposures were to diesel exhaust containing 4.24 mg/m³ particulate matter for 19 h/day, 5 days/week for 120 (hamsters) to 140 (rats) weeks.

Protein, β -glucuronidase activity, and acid phosphatase activity were significantly elevated in BALF obtained from rats exposed to diesel exhaust containing 0.75 or 1.5 mg/m³ particulate matter for 12 mo (Strom, 1984). Exposure for 6 mo resulted in significant increases in acid phosphatase activity at 0.75 mg/m³ and in protein, β -glucuronidase, and acid phosphatase activity at the 1.5 mg/m³ concentration. Exposure at 0.25 mg/m³ particulate matter did not affect the

three indices measured at either time period. The exposures were for 20 h/day, 5.5 days/week for 52 weeks.

Additional biochemical studies (Misirowski et al., 1980) were conducted on laboratory animals exposed under the same conditions and at the same site as reported on by Strom (1984). In most cases, exposures at 0.25 mg/m³ did not cause any significant changes. The DNA content in lung tissue and the rate of collagen synthesis were significantly increased at 1.5 mg/m³ particulate matter after 6 mo. Collagen deposition was not affected. Total lung collagen content increased in proportion to the increase in lung weight. The activity of prolyl hydroxylase was significantly increased at 12 weeks at 0.25 and 1.5 mg/m³; it then decreased with age. Lysal oxidase activity did not change. After 9 mo of exposure, there were significant increases in lung phospholipids in rats and guinea pigs exposed to 0.75 mg/m³ and in lung cholesterol in rats and guinea pigs exposed to 1.5 mg/m³. Pulmonary prostaglandin dehydrogenase activity was stimulated by an exposure at 0.25 mg/m³ but was not affected by exposure at 1.5 mg/m³ (Chaudhari et al., 1980, 1981). Exposures for 12 or 24 weeks resulted in a concentration-dependent lowering of this enzyme activity. Exposure of male rats and guinea pigs at 0.75 mg/m³ for 12 weeks did not cause any changes in glutathione levels of the lung, heart, or liver. Rats exposed for 2 mo at 6 mg/m³ showed a significant depletion of hepatic glutathione, whereas the lung showed an increase of glutathione (Chaudhari and Dutta, 1982). Schneider and Felt (1981) reported that similar exposures did not substantially change adenylate cyclase and guanylate cyclase activities in lung or liver tissue of exposed rats and guinea pigs.

Bhatnagar et al. (1980; see also Pepelko, 1982a) evaluated changes in the biochemistry of lung connective tissue of diesel-exposed rats and mice. The mice were exposed for 8 h/day and 7 days/week for up to 9 mo to exhaust containing 6 mg/m³ particulate matter. Total lung protein content was measured as was labeled proline and labeled leucine. Leucine incorporation is an index of total protein synthesis, although collagen is very low in leucine. Proline incorporation reflects collagen synthesis. Amino acid incorporation was measured in vivo in the rat and in short-term organ culture in mice. Both rats and mice showed a large increase in total protein (41 to 47% in rats), while leucine incorporation declined and proline incorporation was unchanged. These data are consistent with an overall depression of protein synthesis in diesel-exposed animals and also with a relative increase in collagen synthesis compared to other proteins. The increase in collagen synthesis suggested proliferation of connective tissue and possible fibrosis (Pepelko, 1982a).

A number of reports (McClellan et al., 1986; Mauderly et al., 1987a, 1990a; Henderson et al., 1988) have addressed biochemical and cytological changes in lung tissue and BALF of rodents exposed for 7 h/day, 5 days/week for up to 30 mo at concentrations of 0, 0.35, 3.5, or 7.1 mg/m³ particulate matter. At the lowest exposure level (0.35 mg/m³), no biochemical or cytological

changes occurred in the BALF or in lung tissue in either Fischer 344 rats or CD-1 mice. Henderson et al. (1988) provide considerable time-course information on inflammatory events taking place throughout a chronic exposure. A chronic inflammatory response was seen at the two higher exposure levels in both species, as evidenced by increases in inflammatory cells (macrophages and neutrophils), cytoplasmic and lysosomal enzymes (lactate dehydrogenase, glutathione reductase, and β -glucuronidase), and protein (hydroxyproline) in BALF. Analysis of lung tissue indicated similar changes in enzyme levels as well as an increase in total lung collagen content. After 18 mo of exposure, lung tissue glutathione was depleted in a concentration-dependent fashion in rats but was slightly increased in mice. Lavage fluid levels of glutathione and glutathione reductase activity increased in a concentration-dependent manner and were higher in mice than in rats. Rats exposed for 24 mo to diesel exhaust (3.5 mg/m^3 particulate matter) had a fivefold increase in the bronchoconstrictive prostaglandin $\text{PGF}_2\alpha$ and a twofold increase in the inflammatory leukotriene LTB_4 . In similarly exposed mice, there was a twofold increase in both parameters. These investigators concluded that the release of larger amounts of such mediators of inflammation from the alveolar phagocytic cells of rats accounted for the greater fibrogenic response seen in that species.

Biochemical analysis of lung tissue from cats exposed for 124 weeks and held in clean air for an additional 26 weeks indicated increases of lung collagen; this finding was confirmed by an observed increase in total lung wet weight and in connective tissue fibers estimated morphometrically in these cats (Hyde et al., 1985). Exposures were for 7 h/day, 5 days/week at 6 mg/m^3 particulate matter for 61 weeks and at 12 mg/m^3 for weeks 62 to 124.

Heinrich et al. (1995) reported on bronchoalveolar lavage in animals exposed for 24 mo and found exposure-related increases in lactate dehydrogenase, β -glucuronidase, protein, and hydroxyproline in groups exposed to 2.5 or 7 mg/m^3 , although detailed data are not presented. Lavage analyses were not carried out in concurrent studies in mice.

Further effects of exposure to diesel exhaust on pulmonary cytology and lung biochemistry may be found in Section 5.1.2.3.

The pathogenic sequence following the inhalation of diesel exhaust as determined histopathologically and biochemically begins with the phagocytosis of diesel particles by AMs. These activated macrophages release chemotactic factors that attract neutrophils and additional AMs. As the lung burden of diesel particles increases, there is an aggregation of particle-laden AMs in alveoli adjacent to terminal bronchioles, increases in the number of Type II cells lining particle-laden alveoli, and the presence of particles within alveolar and peribronchial interstitial tissues and associated lymph nodes. The neutrophils and macrophages release mediators of inflammation and oxygen radicals that deplete a biochemical defense mechanism of the lung (i.e., glutathione). As will be described later in more detail, other defense mechanisms are affected,

particularly the decreased viability of AMs, which leads to decreased phagocytic activity and death of the macrophage. The latter series of events may result in the presence of pulmonary inflammatory, fibrotic, or emphysematous lesions. The data suggest that there may be a threshold of exposure to diesel exhaust below which adverse structural and biochemical effects may not occur in the lung; however, differences in the anatomy and pathological responses of laboratory animals coupled with their lifespans compared with humans make a determination of human levels of exposure to diesel exhaust without resultant pulmonary injury a difficult and challenging endeavor.

5.1.2.3.4. *Effects on pulmonary defense mechanisms.* The respiratory system has a number of defense mechanisms that negate or compensate for the effects produced by the injurious substances that repeatedly insult the upper respiratory tract, the tracheobronchial airways, and the alveoli. The effects of exposure on the pulmonary defense mechanisms of laboratory animals as well as more details on exposure atmosphere are summarized in Table 5-7 and ranked by cumulative exposure ($C \times T$).

Several studies have been conducted investigating the effect of inhaled diesel exhaust on the deposition and fate of inert tracer particles or diesel particles themselves. Lung clearance of deposited particles occurs in two distinct phases: a rapid phase (hours to days) from the tracheobronchial region via the mucociliary escalator and a much slower phase (weeks to mo) from the nonciliated pulmonary region via primarily but not solely AMs. Battigelli et al. (1966) reported impaired tracheal mucociliary clearance in vitro in excised trachea from rats exposed for single or repeated exposures of 4 to 6 hours at two dilutions of diesel exhaust that resulted in exposures of approximately 8 and 17 mg/m³ diesel particles. The exposure to 17 mg/m³ resulted in decreased clearance after a single exposure as well as after a cumulative exposure of 34 or 100 hours. Clearance was reduced to a lesser extent and in fewer tracheas from animals exposed to 8 mg/m³ for a cumulative exposure of 40 hours. Lewis et al. (1989) found no difference in the

Table 5-7. Effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
ALVEOLAR MACROPHAGE STATUS								
Guinea Pig, Hartley	20 h/day 5.5 days/week 8 weeks	0.25 1.5 0.19 µm MDD	220 1,320	2.9 7.5	— —	— —	No significant changes in absolute numbers of AMs	Chen et. al. (1980)
Rat, F344, M	7 h/day 5 days/week 104 weeks	2.0 0.23–0.36 µm MDD	7,280	11.5	1.5	0.81	Little effect on viability, cell number, oxygen consumption, membrane integrity, lysosomal enzyme activity, or protein content of AMs; decreased cell volume and ruffling of cell membrane and depressed luminescence of AM	Castranova et al. (1985)
Rat, F344, M	20 h/day 5.5 days/week 26, 48, or 52 weeks	0.25 ^a 0.75 ^a 1.5 ^b 0.19 µm MDD	715-8,580	2.9 4.8 7.5	— — —	— — —	AM cell counts proportional to concentration of DPM at 0.75 and 1.5 mg/m ³ ; AM increased in lungs in response to rate of DPM mass entering lung rather than total DPM burden in lung; increased PMNs were proportional to inhaled concentrations and/or duration of exposure; PMNs affiliated with clusters of aggregated AM rather than DPM	Strom (1984) Vostal et al. (1982)
Rat F344/Crl, M, F Mouse, CD, M, F	7 h/day 5 days/week 104 weeks (rat), 78 weeks (mouse)	0.35 3.5 7.0 0.25 µm MDD	1,274 ^c 12,740 ^c 25,480 ^c	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Significant increases of AM in rats and mice exposed to 7.0 mg/m ³ DPM for 24 and 18 mo, respectively, but not at concentrations of 3.5 or 0.35 mg/m ³ DPM for the same exposure durations; PMNs increased in a dose-dependent fashion in both rats and mice exposed to 3.5 or 7.0 mg/m ³ DPM and were greater in mice than in rats	Henderson et al. (1988)
Rat, Wistar, F	18 h/day 5 days/week 24 mo	0.8 2.5 7.1	7,400 21,800 61,700	2.6 8.3 21.2	0.3 1.1 3.4	— — —	Changes in differential cell counts in lung lavage	Heinrich et al. (1995)
Rat, F344/Crl, M	7 h/day 5 days/week 24 mo	3.49	12,704	9.8	1.2	—	Significantly reduced AM in lavage at 24 mo	Mauderly et al. (1990a)

CLEARANCE

Table 5-7. Effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, M, F	7 h/day	0.2	84	—	—	—	Evidence of apparent speeding of tracheal clearance at the 4.5 mg/m ³ level after 1 week of ^{99m} Tc macroaggregated-albumin and reduced clearance of tracer aerosol in each of the three exposure levels at 12 weeks; indication of a lower percentage of ciliated cells at the 1.0 and 4.5 mg/m ³ levels	Wolff and Gray (1980)
	5 days/week	1.0	420	—	—	—		
	12 weeks	4.5	1,890	—	—	—		
		0.25µm MDD						
Rat, Wistar, F	18 h/day	0.8	7,400	2.6	0.3	0.3	Significant increase in clearance half-time of inhaled labeled aerosols in all groups at 3-18 mo.	Heinrich et al. (1995)
	5 days/week	2.5	21,800	8.3	1.2	1.1		
	24 mo	7.1	61,700	21.2	3.8	3.4		
Rat, F344, M, developing 0-6 mo adult 6-12 mo	7 h/day 5 days/week 6 mo	3.55	3,321	7.9	9.5		Clearance of 2 µm, aluminosilicate particles. Half-time significantly increased in adult, not different in developing rats	Mauderly et al. (1987b)
Rat, F344, M, F	7 h/day	0.15	94.5	—	—	—	Lung burdens of DPM were concentration-related; clearance half-time of DPM almost double in 4.1 mg/m ³ group compared to 0.15 mg/m ³ group	Griffis et al. (1983)
	5 days/week	0.94	592	—	—	—		
	18 weeks	4.1	2,583	—	—	—		
		<0.5 µm MDD						
Rat, F344, M	7 h/day 5 days/week 26-104 weeks	2.0 0.23-0.36 µm MDD	1,820-7,280	11.5	1.5	0.8	No difference in clearance of ⁵⁹ Fe ₂ O ₄ particles 1 day after tracer aerosol administration; 120 days after exposure tracer aerosol clearance was enhanced; lung burden of DPM increased significantly between 12 and 24 mo of exposure	Lewis et al. (1989)
Rat, Sprague-Dawley, M	4-6 h/day	0.9	2.5-10,210	—	5.0	0.2	Impairment of tracheal mucociliary clearance in a concentration-response manner	Battigelli et al. (1966)
	7 days/week	8.0		—	2.7	0.6		
	0.1 to 14.3 weeks	17.0		—	8.0	1.0		
Rat, F344, M, F	7 h/day	0.35	1,593	2.9	0.1	—	No changes in tracheal mucociliary clearance after 6, 12, 18, 24, or 30 mo of exposure; increases in lung clearance half-times as early as 6 mo at 7.0 mg/m ³ level and 18 mo at 3.5 mg/m ³ level; no changes seen at 0.35 mg/m ³ level; after 24 mo of diesel exposure, long-term clearance half-times were increased in the 3.5 and 7.0 mg/m ³ groups	Wolff et al. (1987)
	5 days/week	3.5	15,925	16.5	0.3	—		
	130 weeks	7.1	31,850	29.7	0.7	—		
		0.25 µm MDD						

Table 5-7. Effects of exposure to diesel exhaust on the pulmonary defense mechanisms of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344/Crl, M	7 h/day 5 days/week 24 mo	3.49	12,704	9.8	1.2	—	Doubling of long-term clearance half-time for clearance of 1.0 µm aluminosilicate particles. Less effect on clearance in animals with experimentally induced emphysema	Mauderly et al. (1990a)
MICROBIAL-INDUCED MORTALITY								
Mice, CD-1, F	—	—	—	—	—	—	No change in mortality in mice exposed intratracheally to 100 µg of DPM prior to exposure to aerosolized <i>Streptococcus</i> sp.	Hatch et al. (1985)
Mice CD-1, F	7 h/day 5 days/week 4, 12, or 26 weeks	2.0 0.23–0.36 µm MDD	280-1,820	11.5	1.5	0.8	Mortality similar at each exposure duration when challenged with Ao/PR/8/34 influenza virus; in mice exposed for 3 and 6 mo, but not 1 mo, there were increases in the percentages of mice having lung consolidation, higher virus growth, depressed interferon levels, and a fourfold reduction in hemagglutinin antibody levels	Hahon et al. (1985)
Mice, CR/CD-1, F	8 h/day 7 days/week 2 h up to 46 weeks	5.3 to 7.9	11-20,350	19 to 22	1.8 to 3.6	0.9 to 2.8	Enhanced susceptibility to lethal effects of <i>S. pyogenes</i> infections at all exposure durations (2 and 6 h; 8, 15, 16, 307, and 321 days); inconclusive results with <i>S. typhimurium</i> because of high mortality rates in controls; no enhanced mortality when challenged with A/PR8-3 influenza virus	Campbell et al. (1980, 1981)

^aChronic exposure lasted 52 weeks.^bChronic exposure lasted 48 weeks.^cCalculated for 104-week exposure.

DPM = Diesel particulate matter.

AM = Alveolar macrophage.

PMN = Polymorphonuclear leukocyte.

clearance of $^{59}\text{Fe}_3\text{O}_4$ particles (1.5 μm MMAD, σ_g 1.8) 1 day after dosing control and diesel exhaust-exposed rats (2 mg/m^3 , 7 h/day, 5 days/week for 8 weeks).

Wolff et al. (1987) and Wolff and Gray (1980) studied the effects of both subchronic and chronic diesel exhaust exposure on the tracheal clearance of particles. Tracheal clearance assessments were made by measuring the retention of radiolabeled technetium macroaggregated-albumin remaining 1 h after instillation in the distal trachea of rats. In the subchronic studies, rats were exposed to 4.5, 1.0, or 0.2 mg/m^3 particulate matter on a 7 h/day, 5 days/week schedule for up to 12 weeks. After 1 week there was an apparent speeding of tracheal clearance at the 4.5 mg/m^3 exposure level ($p=0.10$), which returned toward baseline after 6 weeks and was slightly below the baseline rate at 12 weeks. In the 1.0 mg/m^3 group, there was a progressive significant reduction in the clearance rate at 6 and 12 weeks of exposure. There was a trend toward reduced clearance in the 0.2 mg/m^3 group. Scanning electron micrographs indicated minimal changes in ciliary morphology; however, there was an indication of a lower percentage of ciliated cells at the 1.0 and 4.5 mg/m^3 levels. In the chronic studies, rats were exposed to 0, 0.35, 3.5, or 7.1 mg/m^3 for 7 h/day, 5 days/week for 30 mo. There were no significant differences in tracheal clearance rates between the control group and any of the exposure groups after 6, 12, 18, 24, or 30 mo of exposure. The preexposure measurements for all groups, however, were significantly lower than those during the exposure period, suggesting a possible age effect. The preexposure value for the 3.5- mg/m^3 group was also significantly lower than the control group.

There is a substantial body of evidence for an impairment of particle clearance from the bronchioloalveolar region of rats following exposure to diesel exhaust. Griffis et al. (1983) exposed rats 7 h/day, 5 days/week for 18 weeks to diesel exhaust at 0.15, 0.94, or 4.1 mg/m^3 particulate matter. Lung burdens of the 0.15, 0.94, and 4.1 mg/m^3 levels were 35, 220, and 1,890 $\mu\text{g}/\text{g}$ lung, respectively, 1 day after the 18-week exposure. The clearance half-time of the DPM was significantly greater, almost double, for the 4.1 mg/m^3 exposure group than for those of the lower exposure groups, 165 ± 8 days versus 99 ± 8 days (0.94 mg/m^3) and 87 ± 28 days (0.15 mg/m^3), respectively.

Chan et al. (1981) showed a dose-related slowing of ^{14}C -diesel particle clearance in rats preexposed to diesel exhaust at 0.25 or 6 mg/m^3 particulate matter for 20 h/day, 7 days/week for 7 to 112 days. Clearance was inhibited in the 6 mg/m^3 group when compared by length of exposure or compared with the 0.25 mg/m^3 or control rats at the same time periods.

Heinrich et al. (1982) evaluated lung clearance in rats exposed for approximately 18 mo at 3.9 mg/m^3 DPM for 7 to 8 h/day, 5 days/week. Following exposure to $^{59}\text{Fe}_2\text{O}_3$ -aerosol, the rats were returned to the diesel exhaust exposure and the radioactivity was measured over the thoracic area at subsequent times. The biological half-life of the iron oxide deposited in the rats' lungs was nearly twice that of controls.

Heinrich also used labeled iron oxide aerosols to study clearance in rats exposed to 0.8, 2.5, or 7 mg/m³ diesel DPM for 24 mo (Heinrich et al., 1995). Clearance measurements were carried out at 3, 12, and 18 mo of exposure. Half-times of clearance were increased in a concentration- and duration-related way in all exposed groups, with a range of a 50% increase in the 0.8 mg/m³ group at 3 mo to an 11-fold increase in the 7 mg/m³ group at 19 mo. The differential cell counts in these animals were stated to have shown clear effects in the 2.5 and 7 mg/m³ groups, but specific information about the changes is not reported.

Wolff et al. (1987) investigated alterations in DPM clearance from the lungs of rats chronically exposed to diesel exhaust at 0, 0.35, 3.5, or 7.0 mg/m³ DPM for 7 h/day, 5 days/week for up to 24 mo. Progressive increases in lung burdens were observed over time in the 3.5 and 7.0 mg/m³ exposure groups. Levels of DPM in terms of milligrams per lung were 0.60, 11.5, and 20.5 after 24 mo of exposure at the 0.35, 3.5, or 7.0 mg/m³ exposure levels, respectively. There were significant increases in 16-day clearance half-times of inhaled radiolabeled particles of ⁶⁷Ga₂O₃ (0.1 µm MMD) as early as 6 mo at the 7.0 mg/m³ level and 18 mo at the 3.5 mg/m³ level; no significant changes were seen at the 0.35 mg/m³ level. Rats inhaled fused aluminosilicate particles (2 µm MMAD) labeled with ¹³⁴Cs after 24 mo of diesel exhaust exposure; long-term clearance half-times were 79, 81, 264, and 240 days for the 0, 0.35, 3.5, and 7.0 mg/m³ groups, respectively. Differences were significant between the control and the 3.5 and 7.0 mg/m³ groups (*p* < 0.01).

Mauderly et al. (1987b) compared the effects of diesel exhaust in the developing lung to the adult lung by exposing groups of male F344 rats to 3.5 mg/m³ for 7 h/day, 5 days/week for 6 mo. One group (adult) was exposed between 6 and 12 mo of age, and the other was exposed beginning in utero and until 6 mo of age. Clearance of an inhaled monodisperse 2 µm aluminosilicate particle was measured after exposure for 6 mo. The clearance half-time of the slow phase was found to be doubled in adult rats compared with age-matched controls and was not significantly affected in developing rat lungs.

Mauderly et al. compared the effects of diesel exhaust in normal lungs with rats in which emphysema had been induced experimentally by instillation of elastase 6 weeks before diesel exhaust exposures. The rats were exposed to 3.5 mg/m³ DPM for 7 h/day, 5 days/week for 24 mo. Measurements included histopathology, clearance, pulmonary function, lung lavage, and immune response. In the rats that were not pretreated with elastase, there was a significant reduction in the number of macrophages recovered by pulmonary lavage in contrast to the increases in macrophages reported by Strom (1984) and Henderson et al. (1988). The half-time of the slow phase of clearance of inhaled, 1 µm, monodisperse particles was doubled in the exposure animals without elastase pretreatment. The elastase pretreatment did not affect clearance in unexposed animals but significantly reduced the effect of diesel. The clearance half-

time was significantly less in elastase-pretreated, diesel-exposed animals than in diesel-exposed normal animals. Many other effects measured in this study were also less affected by diesel exposure in elastase-treated animals. Measurements of lung burden of DPM showed that elastase-pretreated animals accumulated less than half as much DPM mass as normal animals exposed at the same time, suggesting that the difference in effect could be explained by differences in dose to the lung.

Lewis et al. (1989) conducted lung burden and $^{59}\text{Fe}_3\text{O}_4$ tracer studies in rats exposed for 12 and 24 mo to 2 mg/m^3 DPM (7 h/day, 5 days/week). The slope of the Fe_3O_4 clearance curve was significantly steeper than that of the controls, indicating a more rapid alveolar clearance of the deposited $^{59}\text{Fe}_3\text{O}_4$. After 120 days from the inhalation of the tracer particle, 19% and 8% of the initially deposited $^{59}\text{Fe}_3\text{O}_4$ were present in the lungs of control and diesel exhaust-exposed rats, respectively. The lung burden of DPM, however, increased significantly between 12 and 24 mo of exposure (0.52 to 0.97% lung dry weight), indicating a later dose-dependent inhibition of clearance.

Alveolar macrophages, because of their phagocytic and digestive capabilities, are one of the prime defense mechanisms of the alveolar region of the lung against inhaled particles. Thus, characterization of the effects of diesel exhaust on various properties of AMs provides information on the integrity or compromise of a key pulmonary defense mechanism. The physiological viability of AM from diesel-exposed rats was assessed after 2 years of exposure by Castranova et al. (1985). The 7 h/day, 5 days/week exposure at 2 mg/m^3 DPM had little effect on the following: viability, cell number, oxygen consumption, membrane integrity, lysosomal enzyme activity, or protein content of the AM. A slight decrease in cell volume, a decrease in chemiluminescence indicative of a decreased secretion of reactive oxygen species, and a decrease in ruffling of the cell membrane were observed. These findings could be reflective of an overall reduction in phagocytic activity.

Exposure to diesel exhaust has been reported both to increase the number of recoverable AMs from the lung (Strom, 1984; Vostal et al., 1982; Henderson et al., 1988) or to produce no change in numbers (Chen et al., 1980; Castranova et al., 1985). Strom (1984) found that in rats exposed to 0.25 mg/m^3 DPM for 20 h/day, 5.5 days/week for 6 mo or 1 year, as well as in the controls, BAL cells consisted entirely of AMs, with no differences in the cell counts in the lavage fluid. At the higher concentrations, 0.75 or 1.5 mg/m^3 , the count of AM increased proportionally with the exposure concentration; the results were identical for AMs at both 6 and 11 or 12 mo of exposure. The increase in AM counts was much larger after exposure to 1.5 mg/m^3 DPM for 6 mo than after exposure to 0.75 mg/m^3 for 1 year, although the total mass (calculated as $C \times T$) of deposited particulate burden was the same. These data suggested to the authors that the number of lavaged AM was proportional to the mass influx of particles rather than to the actual DPM

burden in the lung. These results further implied that there may be a threshold for the rate of mass influx of DPM into the lungs of rats above which there was an increased recruitment of AMs. Henderson et al. (1988) reported similar findings of significant increases of AMs in rats and mice exposed to 7.1 mg/m³ DPM for 18 and 24 mo, respectively, for 7 h/day, 5 days/week, but not at concentrations of 3.5 or 0.35 mg/m³ for the same exposure durations. Chen et al. (1980), using an exposure regimen of 0.25 and 1.5 mg/m³ DPM for 2 mo and 20 h/day and 5.5 days/week, found no significant changes in absolute numbers of AMs from guinea pig BALF nor did Castranova et al. (1985) in rat BALF following exposure to 2 mg/m³ DPM for 7 h/day, 5 days/week for 2 years.

A similar inflammatory response was noted by Henderson et al. (1988) and Strom (1984), as evidenced by an increased number of PMNs present in BALF from rodents exposed to diesel exhaust. Henderson et al. (1988) found these changes in rats and mice exposed to 7.1 and 3.5 mg/m³ DPM for 7 h/day, 5 days/week. Significant increases in BALF PMNs were observed in mice at 6 mo of exposure and thereafter at the 7.1 and 3.5 mg/m³ exposure levels, but in rats only the 7.1 mg/m³ exposure level showed an increase in BALF PMNs at 6 mo of exposure and thereafter. Significant increases in BALF PMNs occurred in rats at 12, 18, and 24 mo of exposure to 3.5 mg/m³ DPM. Although increases in PMNs were usually greater in mice in terms of absolute numbers, the PMN response in terms of increase relative to controls was only about one-third that of rats. Strom (1984) reported that the increased numbers of PMNs in BALF were proportional to the inhaled concentrations and/or duration of exposure. The PMNs also appeared to be affiliated with clusters of aggregated AMs rather than to the diesel particles per se. Proliferation of Type II cells likewise occurred in response to the formed aggregates of AMs (White and Garg, 1981).

The integrity of pulmonary defense mechanisms can also be ascertained by assessing if exposure to diesel exhaust affects the colonization and clearance of pathogens and alters the challenged animals' response to respiratory tract infections. Campbell et al. (1980, 1981) exposed mice to diesel exhaust followed by infectious challenge with *Salmonella typhimurium*, *Streptococcus pyogenes*, or A/PR8-3 influenza virus and measured microbial-induced mortality. Exposures to the diesel exhaust were to 6 mg/m³ DPM for 8 h/day, 7 days/week for up to 321 days. Exposure to the diesel exhaust resulted in enhanced susceptibility to the lethal effects of *S. pyogenes* infection at all exposure durations (2 h, 6 h; 8, 15, 16, 307, and 321 days). Tests with *S. typhimurium* were inconclusive because of the high mortality rates in the controls. The mice exposed to diesel exhaust did not exhibit an enhanced mortality when challenged with the influenza virus. Hatch et al. (1985) found no changes in the susceptibility of mice to Group C *Streptococcus* sp. infection following intratracheal injection of 100 µg of DPM suspended in unbuffered saline.

Hahon et al. (1985) assessed virus-induced mortality, virus multiplication with concomitant interferon (IFN) levels (lungs and sera), antibody response, and lung histopathology in mice exposed to diesel exhaust prior to infectious challenge with Ao/PR/8/34 influenza virus. Weanling mice were exposed to the diesel exhaust containing 2 mg/m³ DPM for 7 h/day, 5 days/week. In mice exposed for 1, 3, and 6 mo, mortality was similar between the exposed and control mice. In mice exposed for 3 and 6 mo, however, there were significant increases in the percentage of mice having lung consolidation, higher virus growth, depressed interferon levels, and a fourfold reduction in hemagglutinin antibody levels; these effects were not seen after the 1-mo exposure.

The effects of diesel exhaust on the pulmonary defense mechanisms are determined by three critical factors related to exposure: the concentrations of the pollutants, the exposure duration, and the exposure pattern. Higher doses of diesel exhaust as determined by an increase in one or more of these three variables have been reported to increase the numbers of AMs, PMNs, and Type II cells in the lung, whereas lower doses fail to produce such changes. The single most significant contributor to the impairment of the pulmonary defense mechanisms appears to be an excessive accumulation of DPM, particularly as particle-laden aggregates of AMs. Such an accumulation would result from an increase in deposition and/or a reduction in clearance. The deposition of particles does not appear to change significantly following exposure to equivalent diesel exhaust doses over time. Because of the significant nonlinearity in particle accumulation between low and high doses of diesel exhaust exposure, coupled with no evidence of increased particle deposition, an impairment in one or more of the mechanisms of pulmonary defense appears to be responsible for the DPM accumulation and subsequent pathological sequelae. The time of onset of pulmonary clearance impairment was dependent both on the magnitude and on the duration of exposures. For example, for rats exposed for 7 h/day, 5 days/week for 104 weeks, the concentration needed to induce pulmonary clearance impairment appears to lie between 0.35 and 2.0 mg/m³ DPM.

5.1.2.3.5. *Effects on the immune system.* The effects of diesel exhaust on the immune system of guinea pigs were investigated by Dziedzic (1981). Exposures were to 1.5 mg/m³ DPM for 20 h/day, 5.5 days/week for up to 8 weeks. There was no effect of diesel exposure when compared with matched controls for the number of B and T lymphocytes and null cells isolated from the tracheobronchial lymph nodes, spleen, and blood. Cell viability as measured by trypan blue exclusion was comparable between the exposed and control groups. The results of this study and others on the effects of exposure to diesel exhaust on the immune system are summarized in Table 5-8.

Mentnech et al. (1984) examined the effect of diesel exhaust on the immune system of rats. Exposures were to 2 mg/m³ DPM for 7 h/day, 5 days/week for up to 2 years. Rats exposed for 12 and 24 mo were tested for immunocompetency by determining antibody-producing cells in the spleen 4 days after immunization with sheep erythrocytes. The proliferative response of splenic T-lymphocytes to the mitogens concanavalin A and phytohemagglutinin was assessed in rats exposed for 24 mo. There were no significant differences between the exposed and control animals. Results obtained from these two assays indicate that neither humoral immunity (assessed by enumerating antibody-producing cells) nor cellular immunity (assessed by the lymphocyte blast transformation assay) were markedly affected by the exposures.

Bice et al. (1985) evaluated whether or not exposure to diesel exhaust would alter antibody immune responses induced after lung immunization of rats and mice. Exposures were to 0.35, 3.5, or 7.1 mg/m³ DPM for 7 h/day, 5 days/week for 24 mo. Chamber controls and exposed animals were immunized by intratracheal instillation of sheep red blood cells (SRBC) after 6, 12, 18, or 24 mo of exposure. No suppression in the immune response occurred in either species. After 12, 18, and 24 mo of exposure, the total number of anti-SRBC IgM antibody forming cells (AFCs) was elevated in rats, but not in mice, exposed to 3.5 or 7.1 mg/m³ DPM; after 6 mo of exposure, only the 7.1 mg/m³ level was found to have caused this response in rats. The number of AFC per 10⁶ lymphoid cells in lung-associated lymph nodes and the levels of specific IgM, IgG, or IgA in rat sera were not significantly altered. The investigators concluded that the increased cellularity and the presence of DPM in the lung-associated lymph nodes had only a minimal effect on the immune and antigen filtration function of these tissues. Takafuji et al. (1987) evaluated the IgE antibody response of mice inoculated intranasally at intervals of 3 weeks with varying doses of a suspension of DPM in ovalbumin.

Antiovalbumin IgE antibody titers, assayed by passive cutaneous anaphylaxis, were enhanced by doses as low as 1 µg of particles compared with immunization with ovalbumin alone.

The inhalation of diesel exhaust appeared to have only minimal effects on the immune status of rats and guinea pigs. Conversely, intranasally delivered doses as low as 1 µg of DPM exerted an adjuvant activity for IgE antibody production in mice. Further studies of the effects of

Table 5-8. Effects of exposures to diesel exhaust on the immune system of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mouse, BDF1, F	—	—	—	—	—	—	Intranasally delivered doses of DPM as low as 1 µg exerted an adjuvant activity for IgE antibody production	Takafuji et al. (1987)
Guinea Pig, Hartley, M	20 h/day 5.5 days/week 4 or 8 weeks	1.5 0.19 µm MDD	660 or 7,280	7.5	—	—	No alterations in numbers of B, T, and null lymphocytes or cell viability among lymphocytes isolated from tracheobronchial lymph nodes, spleen, or blood	Dziedzic (1981)
Rat, F344, M	7 h/day 5 days/week 52 or 104 weeks	2.0 0.23–0.36 µm MDD	3,640 or 7,280	11.5	1.5	0.8	Neither humoral immunity (assessed by enumerating antibody-producing cells) nor cellular immunity (assessed by the lymphocyte blast transformation assay) were markedly affected	Mentnech et al. (1984)
Rat, F344; Mouse, CD-1	7 h/day 5 days/week 104 weeks	0.35 3.5 7.1 0.25 µm MDD	1,274 12,740 25,480	2.9 16.5 29.7	0.05 0.34 0.68	— — —	Total number of anti-sheep red blood cell IgM AFC in the lung-associated lymph nodes was elevated in rats exposed to 3.5 or 7.0 mg/m ³ DPM (no such effects in mice); total number of AFC per 10 ⁶ lymphoid cells in lung-associated lymph nodes and level of specific IgM, IgG, or IgA in rat sera were not altered	Bice et al. (1985)

DPM = Diesel particulate matter.

AFC = Antibody-forming cells.

diesel exhaust on the immune system are needed to clarify the impact of such variables as route of exposure, species, dose, and atopy.

5.1.2.3.6. *Effects on the liver.* Meiss et al. (1981) examined alterations in the hepatic parenchyma of hamsters by using thin-section and freeze-fracture histological techniques. Exposures to diesel exhaust were for 7 to 8 h/day, 5 days/week, for 5 mo at about 4 or 11 mg/m³ DPM. The livers of the hamsters exposed to both concentrations of diesel exhaust exhibited moderate dilatation of the sinusoids, with activation of the Kupffer cells and slight changes in the cell nuclei. Fatty deposits were observed in the sinusoids, and small fat droplets were occasionally observed in the peripheral hepatocytes. Mitochondria often had a loss of cristae and exhibited a pleomorphic character. Giant microbodies were seen in the hepatocytes, which were moderately enlarged, and gap junctions between hepatocytes exhibited a wide range in structural diversity. The results of this study and others on the effect of exposure of diesel exhaust on the liver of laboratory animals are summarized in Table 5-9.

Green et al. (1983) and Plopper et al. (1983) reported no changes in liver weights of rats exposed to 2 mg/m³ DPM for 7 h/day, 5 days/week for 52 weeks or of cats exposed to 6 to 12 mg/m³, 8 h/day, 7 days/week for 124 weeks.

The use of light and electron microscopy revealed that long-term inhalation of varying high concentrations of diesel exhaust caused numerous alterations to the hepatic parenchyma of guinea pigs. A less sensitive index of liver toxicity, increased liver weight, failed to denote an effect of diesel exhaust on the liver of the rat and cat following long-term exposure to diesel exhaust. These results are too limited to understand potential impacts on the liver.

5.1.2.3.7. *Blood and cardiovascular systems.* Several studies have evaluated the effects of diesel exhaust exposure on hematological and cardiovascular parameters of laboratory animals. These studies are summarized in Table 5-10. Standard hematological indices of toxicological effects on red and white blood cells failed to denote dramatic and consistent responses. Erythrocyte (RBC) counts were reported as being unaffected in cats (Pepelko and Peirano, 1983), rats and monkeys (Lewis et al., 1989), guinea pigs and rats (Penney et al., 1981), and rats (Karagianes et al., 1981); lowered in rats (Heinrich et al., 1982); and elevated in rats (Research Committee for HERP Studies, 1988; Brightwell et al., 1986). Mean corpuscular volume was significantly increased in monkeys, 69 versus 64 (Lewis et al., 1989), and hamsters (Heinrich et al., 1982) and lowered in rats (Research Committee for HERP Studies, 1988). The only other parameters of erythrocyte status and related events were lowered mean corpuscular hemoglobin

Table 5-9. Effects of exposure to diesel exhaust on the liver of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 52 weeks	2.0 0.23–0.36 µm MDD	3,640	12.7	1.6	0.83	No changes in absolute liver weight or liver/body weight ratio	Green et al. (1983)
Hamster, Syrian	7-8 h/day 5 days/week 22 weeks	4.0 8.0 11.0	3,080-9,680	12.0 19.0 25.0	0.5 1.0 1.5	3.0 6.0 7.0	Enlarged sinusoids, with activated Kupffer's cells and slight changes of nuclei; fatty deposits; mitochondria, loss of cristae and pleomorphic character; gap junctions between hepatocytes had wide range in structural diversity	Meiss et al. (1981)
Cat, inbred, M	8 h/day 7 days/week 124 weeks	6.0 ^a 12.0 ^b	41,664 83,328	20.2 33.3	2.7 4.4	2.1 5.0	No change in the absolute liver weight	Plopper et al. (1983)

^a1 to 61 weeks of exposure.^b62 to 124 weeks of exposure.

Table 5-10. Effects of exposure to diesel exhaust on the hematological and cardiovascular systems of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2 0.23–0.36 µm MDD	7,280	11.5	1.5	0.8	Increased MCV	Lewis et al. (1989)
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2 0.23–0.36 µm MDD	7,280	11.5	1.5	0.8	Increase in banded neutrophils; no effect on heart or pulmonary arteries	Lewis et al. (1989) Vallyathan et al. (1986)
Guinea Pig, Hartley, M, F	20 h/day 7 days/week 8 weeks	6.3 ^a 6.8 ^b	7,056 7,616	17.4 16.7	2.3 2.9	2.1 1.9	No effect on heart mass or ECG; small decrease in heart rate (IE only)	Wiester et al. (1980)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 75 weeks	3.9 0.1 µm MDD	10,238-11,700	18.5	1.2	3.1	At 29 weeks, lower erythrocyte count; increased MCV; reduced leukocyte count	Heinrich et al. (1982)
Rat, F344; Guinea Pig, Hartley	20 h/day 5.5 days/week 78 weeks	0.25 0.75 1.5 0.19 µm MDD	2,145 6,435 12,870	3.0 4.8 6.9	0.11 0.27 0.49	— — —	No changes in heart mass or hematology at any exhaust level or duration of exposure in either species	Penney et al. (1981)
Rat, Wistar, M	6 h/day 5 days/week 78 weeks	8.3 0.71 µm MDD	19,422	50.0	4-6	—	3% increase in COHb	Karagianes et al. (1981)
Rat, F3444/Jcl, M, F	16 h/day 6 days/week 130 weeks	0.11 ^c 0.41 ^c 1.08 ^c 2.31 ^c 3.72 ^d 0.1 µm MDD	1,373 5,117 13,478 28,829 46,426	1.23 2.12 3.96 7.10 12.9	0.08 0.26 0.70 1.41 3.00	0.38 1.06 2.42 4.70 4.57	At higher concentrations, RBC, Hb, Hct slightly elevated; MCV and mean corpuscular hemoglobin and concentration were lowered	Research Committee for HERP Studies (1988)
Rat, F344	16 h/day 5 days/week 104 weeks	0.7 2.2 6.6	5,824 18,304 54,912	— — 32.0	— — —	— — —	Increases in RBC, Hb, Hct, and WBC, primarily banded neutrophils; suggestion of an increase in prothrombin time; increased heart/body weight and right ventricular/heart ratios and decreased left ventricular contractility in 6.6 mg/m ³ group	Brightwell et al. (1986)

Table 5-10. Effects of exposure to diesel exhaust on the hematological and cardiovascular systems of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Cat, Inbred, M	8 h/day	6.0 ^e	41,664	20.2	2.7	2.1	Increases in banded neutrophils; significant at 12 mo, but not 24 mo	Pepelko and Peirano (1983)
	7 days/week	12.0 ^f	83,328	33.3	4.4	5.0		
	124 weeks							

^aNonirradiated diesel exhaust.

^bIrradiated diesel exhaust.

^cLight-duty engine.

^dHeavy-duty engine.

^e1 to 61 weeks of exposure.

^f62 to 124 weeks of exposure.

Key: MCV = Mean corpuscular volume.

and mean corpuscular hemoglobin concentration in rats (Research Committee for HERP Studies, 1988), a 3 to 5% increase in carboxyhemoglobin saturation in rats (Karagianes et al., 1981), and a suggestion of an increase in prothrombin time (Brightwell et al., 1986). The biological significance of these findings regarding adverse health effects is deemed to be inconsequential.

Three investigators (Pepelko and Peirano, 1983; Lewis et al., 1989; Brightwell et al., 1986) reported an increase in the percentage of banded neutrophils in cats and rats. This effect was not observed in monkeys (Lewis et al., 1989). The health implications of an increase in abnormal maturation of circulating neutrophils are uncertain but indicate a toxic response of leukocytes following exposures to diesel exhaust. Leukocyte counts were reported to be reduced in hamsters (Heinrich et al., 1982); increased in rats (Brightwell et al., 1986); and unaffected in cats, rats, and monkeys (Pepelko and Peirano, 1983; Research Committee for HERP Studies, 1988; Lewis et al., 1989). These inconsistent findings indicate that the leukocyte counts are more indicative of the clinical status of the laboratory animals than any direct effect of exposure to diesel exhaust.

An important consequence of particle retention in the lungs of exposed subjects can be the development of pulmonary hypertension and cor pulmonale. Such pathology usually arises from pulmonary fibrosis or emphysema obliterating the pulmonary vascular bed or by chronic anoxia. No significant changes in heart mass were found in guinea pigs or rats exposed to diesel exhaust (Wiester et al., 1980; Penney et al., 1981; Lewis et al., 1989). Rats exposed to diesel exhaust showed a greater increase in the medial wall thickness of pulmonary arteries of differing diameters and right ventricular wall thickness; these increases, however, did not achieve statistically significant levels (Vallyathan et al., 1986). Brightwell et al. (1986) reported increased heart/body weight and right ventricular/heart weight ratios and decreased left ventricular contractility in rats exposed to 6.6 mg/m³ DPM for 16 h/day, 5 days/week for 104 weeks.

5.1.2.3.8. Serum chemistry. A number of investigators have studied the effects of exposure to diesel exhaust on serum biochemistry and no consistent effects have been found. Such studies are summarized in Table 5-11.

The biological significance of changes in serum chemistry in female but not male rats exposed at 2 mg/m³ DPM for 7 h/day, 5 days/week for 104 weeks (Lewis et al., 1989) is difficult to interpret. Not only were the effects noted in one sex (females) only, but the serum enzymes, lactate dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT), were elevated in the control group, a circumstance contrary to denoting organ damage in the exposed female rats. The elevations of liver-related

Table 5-11. Effects of chronic exposures to diesel exhaust on serum chemistry of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M, F	7 h/day 5 days/week 104 weeks	2.0 0.23 0.36 µm MDD	7,280	11.5	1.5	0.8	Decreased phosphate, LDH, SGOT, and SGPT; increased sodium in females but not males	Lewis et al. (1989)
Hamster, Syrian, M, F	7-8 h/day 5 days/week 75 weeks	3.9 0.1 µm MDD	10,238-11,700	18.5	1.2	3.1	After 29 weeks, increases in SGOT, LDH, alkaline phosphatase, gamma-glutamyl transferase, and BUN	Heinrich et al. (1982)
Rat, F344/JcL, M, F	16 h/day	0.11 ^a	1,373	1.23	0.08	0.38	Lower cholinesterase activity in males in both the light- and heavy-duty series and elevated gamma globulin and electrolyte levels in males and females in both series	Research Committee for HERP Studies (1988)
	6 days/week	0.41 ^a	5,117	2.12	0.26	1.06		
	130 weeks	1.08 ^a	13,478	3.96	3.96	2.42		
		2.31 ^a	28,829	7.10	7.10	4.70		
		3.72 ^b	46,426	12.9	3.00	4.57		
		0.19–0.28 µm MDD						
Rat, F344; Hamster, Syrian	16 h/day	0.7	5,824	—	—	—	Rats, 6.6 mg/m ³ , reduction in blood glucose, blood proteins, triglycerides, and cholesterol; increase in BUN, alkaline phosphate, and aspartate aminotransferases (SGPT and SGOT); hamsters, 6.6 mg/m ³ , decrease in potassium, LDH, aspartate aminotransferase; increase in albumin and gamma-glutamyl transferase	Brightwell et al. (1986)
	5 days/week	2.2	18,304	—	—	—		
	104 weeks	6.6	54,912	32.0	—	—		
Cat inbred, M	8 h/day	6.0 ^c	41,664	20.2	2.7	2.1	BUN unaltered; SGOT and SGPT unaffected; LHD increase after 1 year of exposure	Pepelko and Peirano (1983)
	7 days/week	12.0 ^d	83,328	33.3	4.4	5.0		
	124 weeks							

^aLight-duty engine.

^bHeavy-duty engine.

^c1 to 61 weeks of exposure.

^d62 to 124 weeks of exposure.

Key: LDH = Lactate dehydrogenase.
 SGOT = Serum glutamic-oxaloacetic transaminase.
 BUN = Blood urea nitrogen.
 SGPT = Serum glutamic-pyruvic transaminase.

serum enzymes in the control versus the exposed female rats appear to be a random event among these aged subjects. The incidence of age-related disease, such as mononuclear cell leukemia, can markedly affect such enzyme levels, seriously compromising the usefulness of a comparison to historical controls. The serum sodium values of 144 versus 148 mmol/L in control and exposed rats, respectively, although statistically different, would have no biological import.

The increased serum enzyme activities, alkaline phosphatase, SGOT, SGPT, gamma-glutamyl transpeptidase, and decreased cholinesterase activity suggest an impaired liver; however, such an impairment was not established histopathologically (Heinrich et al., 1982; Research Committee for HERP Studies, 1988; Brightwell et al., 1986). The increased urea nitrogen, electrolyte levels, and gamma globulin concentration and reduction in total blood proteins are indicative of impaired kidney function. Again there was no histopathological confirmation of impaired kidneys in these studies.

Clinical chemistry studies suggest impairment of both liver and kidney functions in rats and hamsters chronically exposed to high concentrations of diesel exhaust. The absence of histopathological confirmation, the appearance of such effects near the end of the lifespan of the laboratory animal, and the failure to find such biochemical changes in cats exposed to a higher dose, however, tend to discredit the probability of hepatic and renal hazards to humans exposed at atmospheric levels of diesel exhaust.

5.1.2.3.9. *Effects on microsomal enzymes.* Several studies have examined the effects of diesel exhaust exposure on microsomal enzymes associated with the metabolism and possible activation of xenobiotics, especially polynuclear aromatic hydrocarbons. These studies are summarized in Table 5-12. Lee et al. (1980) measured the activities of aryl hydrocarbon hydroxylase (AHH) and epoxide hydrase (EH) in liver, lung, testis, and prostate gland of adult male rats exposed to 6.32 mg/m³ DPM 20 h/day for 42 days. Maximal significant AHH activities (pmol/min/mg microsomal protein) occurred at different times during the exposure period, and differences between controls and exposed rats, respectively, were as follows: prostate 0.29 versus 1.31, lung 3.67 versus 5.11, and liver 113.9 versus 164.0. There was no difference in AHH activity in the testis between exposed and control rats. Epoxide hydrase activity was not significantly different from control values for any of the organs tested.

Pepelko and Peirano (1983) found no statistical differences in liver microsomal cytochrome P448-450 levels and liver microsomal AHH between control and diesel-exposed mice either at 6 and 8 mo of exposure. Small differences were noted in the lung microsomal AHH activities, but these were believed to be artifactual differences, due to increases in

Table 5-12. Effects of chronic exposures to diesel exhaust on microsomal enzymes of laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, F344, M	—	—	—	—	—	—	Intratracheal administration of DPM extract required doses greater than 6 mg/m ³ before the lung AHH was barely doubled; liver AHH activity was unchanged	Chen (1986)
Mouse, CD-1, F	7 h/day 5 days/week 4 weeks	2.0 0.2–0.36 µm MDD	280	11.5	1.5	0.8	Mice inoculated intranasally with influenza virus had smaller increases in ethylmorphine demethylase activity on days 2 to 4 postvirus infection and abolition of day 4 postinfection increase in NADPH-dependent cytochrome c reductase	Rabovsky et al. (1986)
Rat, Sprague-Dawley, M	20 h/day 7 days/week 1-7 weeks	6.3	882-6,174	17.4	2.3	2.1	AHH induction occurred in lung, liver, and prostate gland but not in testes; maximum significant activities occurred at different times; liver has greatest overall activity, percent increase highest in prostate; epoxide hydrolase activity was unaffected	Lee et al. (1980)
Rat, F344, M	20 h/day	0.75	330-6,435	4.8	—	—	Inhalation exposure had no significant effect on liver AHH activity; lung AHH activity was slightly reduced after 6-mo exposure to 1.5 mg/m ³ DPM; an ip dose of DP extract, estimated to be equivalent to inhalation exposure, had no effect on AHH activity in liver and lungs; cyt. P-450 was unchanged in lungs and liver following inhalation or ip administration	Chen and Vostal (1981)
	5.5 days/week	1.5		7.5	—	—		
	4, 13, 26, or 39 weeks	0.19 µm MDD	330-6,435	4.8	—	—		
	20 h/day 5.5 days/week 4, 13, 26, or 39 weeks	0.75 1.5 0.19 µm MDD		7.5	—	—		
Rat, F344, F	7 h/day 5 days/week 12, 26, or 104 weeks	2.0 0.23-0.36 µm MDD	840-7,280	11.5	1.5	0.8	No effect on B[a]P hydrolase or 7-ethoxycoumarin deethylase activities in the liver	Rabovsky et al. (1984)
Rat, F344, M	20 h/day	0.25	220-8,745	2.9	—	—	After 8 weeks, no induction of cyt. P-450, cyt. P-448, or NADPH-dependent cyt. c reductase; after 1 year of exposure, liver microsomal oxidation of B[a]P was not increased; 1 year of exposure to either 0.25 or 1.5 mg/m ³ DPM impaired lung microsomal metabolism of B[a]P	Navarro et al. (1981)
	5.5 days/week 8-53 weeks	1.5 0.19 µm MDD		7.5	—	—		

Table 5-12. Effects of chronic exposures to diesel exhaust on microsomal enzymes of laboratory animals (continued)

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mouse, A/J, M	8 days/week 7 days/week 26 or 35 weeks	6.0	17.4	17.4	2.3	2.1	No differences in lung and liver AHH activities and liver P-448, P-450 levels	Pepelko and Peirano (1983)

AHH = Aryl hydrocarbon hydroclase.
B[a]P = Benzo[a]pyrene.

nonmicrosomal lung protein present in the microsomal preparations. Exposures to 6 mg/m³ DPM were for 8 h/day, 7 days/week.

Rabovsky et al. (1984) investigated the effect of chronic exposure to diesel exhaust on microsomal cytochrome P450-associated benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin deethylase activities in rat lung and liver. Male rats were exposed for 7 h/day, 5 days/week for 104 weeks to 2 mg/m³ DPM. The exposure had no effect on B[*a*]P hydroxylase or 7-ethoxycoumarin deethylase activities in lung or liver. In related studies, Rabovsky et al. (1986) examined the effects of diesel exhaust on vitally induced enzyme activity and interferon production in female mice. The mice were exposed for 7 h/day, 5 days/week for 1 month to diesel exhaust diluted to achieve a concentration of 2 mg/m³ DPM. After the exposure, the mice were inoculated intranasally with influenza virus. Changes in serum levels of interferon and liver microsomal activities of 7-ethoxycoumarin, ethylmorphine demethylase, and nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome c reductase were measured. In the absence of viral inoculation, exposure to diesel exhaust had no significant effects on the activity levels of the two liver microsomal monooxygenases and NADPH-dependent cytochrome c reductase. Exposure to diesel exhaust produced smaller increases in ethylmorphine demethylase activity on days 2 to 4 postvirus infection and also abolished the day 4 postinfection increase in NADPH-dependent cytochrome c reductase when compared with nonexposed mice. These data suggested to the authors that the relationship that exists between metabolic detoxification and resistance to infection in unexposed mice was altered during a short-term exposure to diesel exhaust.

Chen and Vostal (1981) measured the activity of AHH and the content of cytochrome P450 in the lungs and livers of rats exposed by inhalation or intraperitoneal (i.p.) injection of a dichloromethane extract of DPM. In the inhalation exposures, the exhaust was diluted to achieve concentrations of 0.75 or 1.5 mg/m³ DPM, and the exposure regimen was 20 h/day, 5.5 days/week for up to 9 mo. The concentration of total hydrocarbons and particle-phase hydrocarbons was not reported. Parenteral administration involved repeated i.p. injections at several dose levels for 4 days. Inhalation exposure had no significant effect on liver microsomal AHH activity; however, lung AHH activity was slightly reduced after 6 mo exposure to 1.5 mg/m³. An i.p. dose of DPM extract, estimated to be equivalent to the inhalation exposure, had no effect on AHH activity in liver or lungs. No changes were observed in cytochrome P450 contents in lungs or liver following inhalation exposure or i.p. treatment. Direct intratracheal administration of a dichloromethane DPM extract required doses greater than 6 mg/kg body weight before the activity of induced AHH in the lung was barely doubled; liver AHH activity remained unchanged (Chen, 1986).

In related studies, Navarro et al. (1981) evaluated the effect of exposure to diesel exhaust on rat hepatic and pulmonary microsomal enzyme activities. The same exposure regimen was employed (20 h/day, 5.5 days/week, for up to 1 year), and the exhaust was diluted to achieve concentrations of 0.25 and 1.5 mg/m³ DPM (a few studies were also conducted at 0.75 mg/m³). After 8 weeks of exposure, there was no evidence for the induction of cytochrome P450, cytochrome P448, or NADPH-dependent cytochrome c reductase in rat liver microsomes. One year of exposure had little, if any, effect on the hepatic metabolism of B[a]P. However, 1 year of exposure to 0.25 and 1.5 mg/m³ significantly impaired the ability of lung microsomes to metabolize B[a]P (0.15 and 0.02 nmole/30 min/mg protein, respectively, versus 0.32 nmole/30 min/mg protein for the controls).

There are conflicting results regarding the induction of microsomal AHH activities in the lungs and liver of rodents exposed to diesel exhaust. One study reported induced AHH activity in the lungs, liver, and prostate of rats exposed to diesel exhaust containing 6.32 mg/m³ DPM for 20 h/day for 42 days; however, no induction of AHH was observed in the lungs of rats and mice exposed to 6 mg/m³ DPM for 8 h/day, 7 days/week for up to 8 mo or to 0.25 to 2 mg/m³ for periods up to 2 years. Exposure to diesel exhaust has not been shown to produce adverse effects on microsomal cytochrome P450 in the lungs or liver of rats or mice. The weight of evidence suggests that the absence of enzyme induction in the rodent lung exposed to diesel exhaust is caused either by the unavailability of the adsorbed hydrocarbons or by their presence in insufficient quantities for enzyme induction.

5.1.2.3.10. *Effects on behavior and neurophysiology.* Studies on the effects of exposure to diesel exhaust on the behavior and neurophysiology of laboratory animals are summarized in Table 5-13. Laurie et al. (1978) and Laurie et al. (1980) examined behavioral alterations in adult and neonatal rats exposed to diesel exhaust. Exposure for 20 h/day, 7 days/week, for 6 weeks to exhaust containing 6 mg/m³ DPM produced a significant reduction in adult spontaneous locomotor activity (SLA) and in neonatal pivoting (Laurie et al., 1978). In a follow-up study, Laurie et al. (1980) found that shorter exposure (8 h/day) to 6 mg/m³ DPM also resulted in a reduction of SLA in adult rats. Laurie et al. (1980) conducted additional behavioral tests on adult rats exposed during their neonatal period. For two of three exposure situations (20 h/day for 17 days postparturition, or 8 h/day for the first 28 or 42 days postparturition), significantly lower SLA was observed in the majority of the tests conducted on the adults after week 5 of measurement. When compared with control rats, adult 15-month-old rats that had been exposed as neonates (20 h/day for 17 days) also exhibited a significantly slower rate of acquisition of a bar-pressing task to obtain food. The investigators noted that the evidence was insufficient to determine whether

Table 5-13. Effects of chronic exposures to diesel exhaust on behavior and neurophysiology

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat, Sprague-Dawley, M	8 h/day 7 days/week 1-4 weeks	6	336-1,344	19	2.5	1.8	Somatosensory and visual evoked potentials revealed longer pulse latencies in pups exposed neonatally	Laurie and Boyes (1980, 1981)
Rat, Sprague-Dawley, F	20 h/day 7 days/week 6 weeks	6	5,040	19	2.5	1.8	Reduction in adult SLA and in neonatal pivoting	Laurie et al. (1978)
Rat, Sprague-Dawley, F	8 or 20 h/day 7 days/week 3, 4, 6, or 16 weeks	6	1,008-13,440	19	2.5	1.8	Reduction in SLA in adults; neonatal exposures for 20 or 8 h/day caused reductions in SLA. Neonatal exposures for 20 h/day for 17 days resulted in a slower rate of a bar-pressing task to obtain food	Laurie et al. (1980)

SLA = Spontaneous locomotor activity.

the differences were the result of a learning deficit or due to some other cause (e.g., motivational or arousal differences).

These data are difficult to interpret in terms of health hazards to humans under ambient environmental conditions because of the high concentration of diesel exhaust to which the laboratory rats were exposed. Additionally, there are no further concentration-response studies to assess at what exposure levels these observed results persist or abate. A permanent alteration in both learning ability and activity resulting from exposures early in life is a health hazard whose significance to humans should be pursued further.

Neurophysiological effects from exposure to diesel exhaust were investigated in rats by Laurie and Boyes (1980, 1981). Rats were exposed to diluted diesel exhaust containing 6 mg/m³ DPM for 8 h/day, 7 days/week from birth up until 28 days of age. Somatosensory evoked potential, as elicited by a 1 mA electrical pulse to the tibial nerve in the left hind limb, and visual evoked potential, as elicited by a flash of light, were the end points tested. An increased pulse latency was reported for the rats exposed to diesel exhaust, and this was thought to be caused by a reduction in the degree of nerve myelination. There was no neuropathological examination, however, to confirm this supposition.

Based on the data presented, it is not possible to specify the particular neurological impairment(s) induced by the exposure to diesel exhaust. Again, these results occurred following exposure to a high level of diesel exhaust and no additional concentration-response studies were performed.

5.1.2.3.11. *Effects on reproduction and development.* Studies of the effects of exposure to diesel exhaust on reproduction and development are summarized in Table 5-14. Twenty rats were exposed 8 h/day on days 6 through 15 of gestation to diluted diesel exhaust containing 6 mg/m³ DPM (Werchowski et al., 1980a,b; Pepelko and Peirano, 1983). There were no signs of maternal toxicity or decreased fertility. No skeletal or visceral teratogenic effects were observed in 20-day-old fetuses (Werchowski et al., 1980a). In a second study, 42 rabbits were exposed to 6 mg/m³ DPM for 8 h/day, on gestation days 6 through 18. No adverse effects on body weight gain or fertility were seen in the does exposed to diesel exhaust. No visceral or skeletal developmental abnormalities were observed in the fetuses (Werchowski et al., 1980b).

Pepelko and Peirano (1983) evaluated the potential for diesel exhaust to affect reproductive performance in mice exposed from 100 days prior to exposure throughout maturity of the F₂ generation. The mice were exposed for 8 h/day, 7 days/week to 12 mg/m³ DPM. In general, treatment-related effects were minimal. Some differences in organ and body weights

Table 5-14. Effects of chronic exposures to diesel exhaust on reproduction and development in laboratory animals

Species/sex	Exposure period	Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Mouse, [C57BL]/6XC3H]F ₁ , M	5 days	50, 100, or 200 mg/kg in corn oil; i.p. injection	—	—	—	—	Dose-related increase in sperm abnormalities; decrease in sperm number at highest dose; testicular weights unaffected	Quinto and De Marinis (1984)
Rat, Sprague-Dawley, F	8 h/day 7 days/week 1.7 weeks	6	571	20	2.7	2.1	No signs of maternal toxicity or decreased fertility; no skeletal or visceral teratogenic effects in 20-day-old fetuses	Werchowski et al. (1980a) Pepelko and Peirano (1983)
Rabbit, New Zealand Albino, F	8 h/day 7 days/week 1.9 weeks	6	638	20	2.7	2.1	No adverse effects on maternal weight gain or fertility; no skeletal or visceral teratogenic effects in the fetuses	Werchowski et al. (1980a) Pepelko and Peirano (1983)
Monkey, Cynomolgus, M	7 h/day 5 days/week 104 weeks	2	7,280	11.5	1.5	0.8	No effects on sperm motility, velocity, density, morphology, or incidence of abnormalities	Lewis et al. (1989)
Mouse, A/Strong, M	8 h/day 7 days/week 31 or 38 weeks	6	10,416-12,768	20	2.7	2.1	No effect on sperm morphology; high rate of spontaneous sperm abnormalities may have masked small effects	Pereira et al. (1981)
Mouse, CD-1, M, F	8 h/day 7 days/week 6 to 28 weeks	12	4,032-18,816	33	4.4	5.0	Overall fertility and survival rates were unaffected in the three-generation reproductive study; only consistent change noted, an increase in lung weights, was diagnosed as anthracosis	Pepelko and Peirano (1983)

were noted, but overall fertility and survival rates were not altered by exposure to diesel exhaust. The only consistent change, an increase in lung weights, was accompanied by a gross pathological diagnosis of anthracosis. These data denoted that exposure to diesel exhaust at a concentration of 12 mg/m³ did not affect reproduction. See Section 5.3, which reports a lack of effects of exposure to diesel exhaust on rat lung development (Mauderly et al., 1987b).

Several studies have evaluated the effect of exposure to diesel exhaust on sperm. Lewis et al. (1989) found no adverse sperm effects (sperm motility, velocity, densities, morphology, or incidence of abnormal sperm) in monkeys exposed for 7 h/day, 5 days/week, for 104 weeks to 2 mg/m³ DPM. In another study in which A/Strong mice were exposed to diesel exhaust containing 6 mg/m³ DPM for 8 h/day for 31 or 38 weeks, no significant differences were observed in sperm morphology between exposed and control mice (Pereira et al., 1981). It was noted, however, that there was a high rate of spontaneous sperm abnormalities in this strain of mice, and this may have masked any small positive effect. Quinto and De Marinis (1984) reported a statistically significant and dose-related increase in sperm abnormalities in mice injected intraperitoneally for 5 days with 50, 100, or 200 mg/kg of DPM suspended in corn oil.

A significant decrease in sperm number was seen at the highest dose, but testicular weight was unaffected by the treatment.

No teratogenic, embryotoxic, fetotoxic, or female reproductive effects were observed in mice, rats, or rabbits at exposure levels up to 12 mg/m³ DPM. Effects on sperm morphology and number were reported in hamsters and mice exposed to high doses of DPM; however, no adverse effects were observed in sperm obtained from monkeys exposed at 2 mg/m³ for 7 h/day, 5 days/week for 104 weeks. Concentrations of 12 mg/m³ DPM did not affect male rat reproductive fertility in the F₀ and F₁ generation breeders. Thus, exposure to diesel exhaust would not appear to be a reproductive or developmental hazard.

5.2. COMPARISON OF HEALTH EFFECTS OF FILTERED AND UNFILTERED DIESEL EXHAUST

In four chronic toxicity studies of diesel exhaust, the experimental protocol included exposing test animals to exhaust containing no particles. Comparisons were then made between the effects caused by whole, unfiltered exhaust and those caused by the gaseous components of the exhaust. Concentrations of components of the exposure atmospheres in these four studies are given in Table 5-15.

Heinrich et al. (1982) compared the toxic effects of whole and filtered diesel exhaust on hamsters and rats. Exposures were for 7 to 8 h/day and 5 days/week. Rats exposed for 24 mo to either whole or filtered exhaust exhibited no significant changes in respiratory frequency,

Table 5-15. Composition of exposure atmospheres in studies comparing unfiltered and filtered diesel exhaust^a

Species/sex	Exposure ^b period		Particles (mg/m ³)	C × T (mg·h/m ³)	CO (ppm)	NO ₂ (ppm)	SO ₂ (ppm)	Effects	Study
Rat Wistar, F; Hamster, Syrian	7 h/day 5 days/week 104 weeks	UF F C	3.9 — —	14,196	18.5 18.0 —	1.2 1.0 —	3.1 2.8 —	No effect on pulmonary function or heart rate in rats; increases in pulmonary adenomatous proliferations in hamsters, UF significantly higher than F or C	Heinrich et al. (1982)
Rat, F344, F	8 h/day 7 days/week 104 weeks	UF F ^c C	4.9 — —	28,538	7.0 — —	1.8 — —	13.1 — —	Body weight decrease after 6 mo in UF, 18 mo in F; lung/body rate weight rate higher in both groups at 24 mo; at 2 years, fibrosis and epithelial hyperplasia in lungs of UF; nominal lung and spleen histologic changes	Iwai et al. (1986)
Rat, F344, M, F; Hamster, Syrian, M, F	16 h/day 5 days/week 104 weeks	UF UF UF F ^d C	0.7 2.2 6.6 — —	5,824 18,304 54,912	— — 32.0 32.0 1.0	— — — — —	— — — — —	UF: elevated red and white cell counts, hematocrit and hemoglobin; increased heart/body weight and right ventricular/heart weight ratios; lower left ventricular contractility; changes in blood chemistry; obstructive and restrictive lung disease; F: no effects	Brightwell et al. (1986)
Rat, Wistar, F; Hamster, Syrian, F; Mouse NMRI, F	19 h/day 5 days/week 120 to 140 weeks	UF F ^d C	4.24 — —	48,336 56,392	12.5 11.1 0.16	1.5 1.2 —	3.1 1.02 —	UF: decreased body wt in rats and mice but not hamsters; increased mortality, mice only; decreased lung compliance and increased airway resistance, rats and hamsters; species differences in lung lavage enzymes and cell counts and lung histopathology and collagen content, most pronounced in rats; F: no effect on glucose-6-phosphate dehydrogenase, total protein, and lung collagen	Heinrich et al. (1986a)
Mouse, NMRI, F, C57BL/6N, F	18 h/day 5 days/week 23 mo (NMRI) 24 mo (C57BL/6N)	UF F C	4.5 0.01 0.01	40,365	14.2 14.2 0.2	2.3 2.9 0.01	2.8 2.4 0.1	UF: increased lung wet weight starting at 3 mo F: no noncancer effects reported	Heinrich et al. (1995)

^aMean values.

^bUF = Unfiltered whole exhaust, F = Filtered exhaust, C = Control.

^cReported to have the same component concentrations as the unfiltered, except particles were present in undetectable amounts.

^dConcentrations reported for high concentration level only.

respiratory minute volume, compliance or resistance as measured by a whole-body plethysmography, or heart rate. In the hamsters, histological changes (adenomatous proliferations) were seen in the lungs of animals exposed to either whole or filtered exhaust; however, in all groups exposed to the whole exhaust the number of hamsters exhibiting such lesions was significantly higher than for the corresponding groups exposed to filtered exhaust or clean air. Severity of the lesions was, however, not reported.

In a second study, Heinrich et al. (1986a, see also Stöber, 1986) compared the toxic effects of whole and filtered diesel exhaust on hamsters, rats, and mice. The test animals (96 per test group) were exposed for 19 h/day, 5 days/week for 120 (hamsters and mice) or 140 (rats) weeks. Body weights of hamsters were unaffected by either exposure. Body weights of rats and mice were reduced by the whole exhaust but not by the filtered exhaust. Exposure-related higher mortality rates occurred in mice after 2 years of exposure to whole exhaust. After 1 year of exposure to the whole exhaust, hamsters exhibited increased lung weights, a significant increase in airway resistance, and a nonsignificant reduction in lung compliance. For the same time period, rats exhibited increased lung weights, a significant decrease in dynamic lung compliance, and a significant increase in airway resistance. Test animals exposed to filtered exhaust did not exhibit such effects. Histopathological examination indicated that different levels of response occurred in the three species. In hamsters, filtered exhaust caused no significant histopathological effects in the lung; whole exhaust caused thickened alveolar septa, bronchioloalveolar hyperplasia, and emphysematous lesions. In mice, whole exhaust, but not filtered exhaust, caused multifocal bronchioloalveolar hyperplasia, multifocal alveolar lipoproteinosis, and multifocal interstitial fibrosis. In rats, there were no significant morphological changes in the lungs following exposure to filtered exhaust. In rats exposed to whole exhaust, there were severe inflammatory changes in the lungs, thickened alveolar septa, foci of macrophages, crystals of cholesterol, and hyperplastic and metaplastic lesions. Biochemical studies of lung lavage fluids of hamsters and mice indicated that exposure to filtered exhaust caused fewer changes than did exposure to whole exhaust. The latter produced significant increases in lactate dehydrogenase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, total protein, protease (pH 5.1), and collagen. The filtered exhaust had a slight but nonsignificant effect on G6P-DH, total protein, and collagen. Similarly, cytological studies showed that while the filtered exhaust had no effect on differential cell counts, the whole exhaust resulted in an increase in leukocytes ($161 \pm 43.3/\mu\text{L}$ versus $55.7 \pm 12.8/\mu\text{L}$ in the controls), a decrease in AM (30.0 ± 12.5 versus $51.3 \pm 12.5/\mu\text{L}$ in the controls), and an increase in granulocytes (125 ± 39.7 versus $1.23 \pm 1.14/\mu\text{L}$ in the controls). All values presented for this study are the mean with its standard deviation. The differences were significant for each cell type. There was also a small increase in lymphocytes (5.81 ± 4.72 versus $3.01 \pm 1.23/\mu\text{L}$ in the controls).

Iwai et al. (1986) exposed rats (24 per group) to whole or filtered diesel exhaust 8 h/day, 7 days/week for 24 mo. The whole exhaust was diluted to achieve a concentration of 4.9 ± 1.6 mg/m³ DPM. Body weights in the whole exhaust group began to decrease after 6 mo and in both exposed groups began to decrease after 18 mo, when compared with controls. Lung-to-body weight ratios of the rats exposed to the whole exhaust showed a significant increase ($p < 0.01$) after 12 mo in comparison with control values. Spleen-to-body weight ratios of both exposed groups were higher than control values after 24 mo. After 6 mo of exposure to whole exhaust, DPM accumulated in AM, and Type II cell hyperplasia was observed. After 2 years of exposure, the alveolar walls had become fibrotic with mast cell infiltration and epithelial hyperplasia. In rats exposed to filtered exhaust, after 2 years there were only minimal histologic changes in the lungs, with slight hyperplasia and stratification of bronchiolar epithelium and infiltration of atypical lymphocytic cells in the spleen.

Brightwell et al. (1986) evaluated the toxic effects of whole and filtered diesel exhaust on rats and hamsters. Three exhaust dilutions were tested, producing concentrations of 0.7, 2.2, and 6.6 mg/m³ DPM. The test animals (144 rats and 312 hamsters per exposure group) were exposed for five 16-h periods per week for 2 years. The four exposure types were gasoline, gasoline catalyst, diesel, and filtered diesel. The results presented were limited to statistically significant differences between exhaust-exposed and control animals. The inference from the discussion section of the paper was that there was a minimum of toxicity in the animals exposed to filtered diesel exhaust: "It is clear from the results presented that statistically significant differences between exhaust-exposed and control animals are almost exclusively limited to animals exposed to either gasoline or unfiltered diesel exhaust." Additional results are described in Section 5.4.

Heinrich et al. (1995) exposed female NMRI and C57BL/6N mice to a diesel exhaust dilution that resulted in a DPM concentration of 4.5 mg/m³ and to the same dilution after filtering to remove the particles. This study is focused on the carcinogenic effects of diesel particle exposure, and inadequate information was presented to compare noncancer effects in filtered versus unfiltered exhaust.

A comparison of the toxic responses in laboratory animals exposed to whole exhaust or filtered exhaust containing no particles demonstrates across studies that when the exhaust is sufficiently diluted to limit the concentrations of gaseous irritants (NO₂ and SO₂), irritant vapors (aldehydes), CO, or other systemic toxicants, the diesel particles are the prime etiologic agents of noncancer health effects, although additivity or synergism with the gases cannot be ruled out. These toxic responses are both functional and pathological and represent cascading sequelae of lung pathology based on concentration and species. The diesel particles plus gas exposures produced biochemical and cytological changes in the lung that are much more prominent than those evoked by the gas phase alone. Such marked differences between whole and filtered diesel

exhaust are also evident from general toxicological indices, such as decreases in body weight and increases in lung weights, pulmonary function measurements, and pulmonary histopathology (e.g., proliferative changes in Type II cells and respiratory bronchiolar epithelium, fibrosis). Hamsters, under equivalent exposure regimens, have lower levels of retained DPM in their lungs than rats and mice do and, consequently, less pulmonary function impairment and pulmonary pathology. These differences may result from lower DPM inspiration and deposition during exposure, greater DPM clearance, or lung tissue less susceptible to the cytotoxicity of deposited DPM.

5.3. INTERACTIVE EFFECTS OF DIESEL EXHAUST

A multitude of factors may influence the susceptibility to exposure to diesel exhaust as well as the resulting response. Some of these have already been discussed in detail (e.g., the composition of diesel exhaust and concentration-response data); others will be addressed in this section (e.g., the interaction of diesel exhaust with factors particular to the exposed individual and the interaction of diesel exhaust components with other airborne contaminants).

Mauderly et al. (1990a) compared the susceptibility of normal rats and rats with preexisting laboratory-induced pulmonary emphysema exposed for 7 h/day, 5 days/week for 24 mo to diesel exhaust containing 3.5 mg/m³ DPM or to clean air (controls). Emphysema was induced in one-half of the rats by intratracheal instillation of elastase 6 weeks before exhaust exposure. Measurements included lung burdens of diesel particles, respiratory function, bronchoalveolar lavage, clearance of radiolabeled particles, pulmonary immune responses, lung collagen, excised lung weight and volume, histopathology, and mean linear intercept of terminal air spaces. None of the data for the 63 parameters measured suggest that rats with emphysematous lungs were more susceptible than rats with normal lungs to the effects of diesel exhaust exposure. In fact, each of the 14 emphysema-exhaust interactions detected by statistical analysis of variance indicated that emphysema acted to reduce the effects of diesel exhaust exposure. Diesel particulate matter accumulated much less rapidly in the lungs of emphysematous rats than in those of normal rats. The mean lung burdens of DPM in the emphysematous rats were 39, 36, and 37% of the lung burdens of normal rats at 12, 18, and 24 mo, respectively. No significant interactions were observed among lung morphometric parameters. Emphysema prevented the exhaust-induced increase for three respiratory indices of expiratory flow rate at low lung volumes, reduced the exhaust-induced increase in nine lavage fluid indicators of lung damage, prevented the expression of an exhaust-induced increase in lung collagen, and reduced the exhaust-induced delay in DPM clearance.

Mauderly et al. (1987b) evaluated the relative susceptibility of developing and adult rat lungs to damage by exposure to diesel exhaust. Rats (48 per test group) were exposed to diesel exhaust containing 3.5 mg/m³ DPM and about 0.8 ppm NO₂. Exposures were for 7 h/day,

5 days/week through gestation to the age of 6 mo, or from the age of 6 to 12 mo. Comparative studies were conducted on respiratory function, immune response, lung clearance, airway fluid enzymes, protein and cytology, lung tissue collagen, and proteinases in both age groups. After the 6-month exposure, adult rats, compared with controls, exhibited (1) more focal aggregates of particle-containing AMs in the alveolar ducts near the terminal bronchioles, (2) a sixfold increase in the neutrophils (as a percentage of total leukocytes) in the airway fluids, (3) a significantly higher number of total lymphoid cells in the pulmonary lymph nodes, (4) delayed clearance of diesel particles and radiolabeled particles ($t_{1/2}$ = 90 days versus 47 days for controls), and (5) increased lung weights. These effects were not seen in the young rats. On a weight for weight (milligrams of DPM per gram of lung) basis, DPM accumulation in the lungs was similar in young and adult rats immediately after the exposure. During the 6-month postexposure period, DPM clearance was much more rapid in the neonatal rats, approximately 2.5-fold. During postexposure, diesel particle-laden macrophages became aggregated in the neonatal rats, but these aggregations were located primarily in a subpleural position. The authors concluded that exposure to diesel exhaust, using pulmonary function, structural (qualitative or quantitative) biochemistry as the indices, did not affect the developing rat lung more severely than the adult rat lung.

As a result of the increasing trend of using diesel-powered equipment in coal mining operations and the concern for adverse health effects in coal miners exposed to both coal dust or coal mine dust and diesel exhaust, Lewis et al. (1989) and Karagianes et al. (1981) investigated the interaction of coal dust and diesel exhaust. Lewis et al. (1989) exposed rats, mice, and cynomolgus monkeys to (1) filtered ambient air, (2) 2 mg/m³ DPM, (3) 2 mg/m³ respirable coal dust, and (4) 1 mg/m³ of both DPM and respirable coal dust. Gaseous and vapor concentrations were identical in both diesel exhaust exposures. Exposures were for 7 h/day, 5 days/week for up to 24 mo. Synergistic effects between diesel exhaust and coal dust were not demonstrated; additive toxic effects were the predominant effects noted.

Karagianes et al. (1981) exposed rats (24 per group) to diesel exhaust containing 8.3 mg/m³ of DPM alone or in combination with about 6 mg/m³ of coal dust. No synergistic effects were found between diesel exhaust and coal dust; additive effects in terms of visual dust burdens in necropsied lungs were related to dose (i.e., length of exposure and airborne particulate concentrations).

The health effects of airborne contaminants from sources other than diesel engines may be altered in the presence of DPM by their adsorption onto the diesel particles. When adsorbed onto diesel particles, the gases and vapors can be transported and deposited deeper into the lungs, and because they are more concentrated on the particle surface, the resultant cytotoxic effects or physiological responses may be enhanced. Nitrogen dioxide adsorbed onto carbon particles

caused pulmonary parenchymal lesions in mice, whereas NO₂ alone produced edema and inflammation but no lesions (Boren, 1964). Exposure to formaldehyde and acrolein adsorbed onto carbon particles (1 to 4 µm) resulted in the recruitment of PMNs to tracheal and intrapulmonary epithelial tissues but not when the aldehydes were tested alone (Kilburn and McKenzie, 1978).

There is no direct evidence that diesel exhaust interacts with other substances in an exposure environment or the physiological status of the exposed subject other than impaired resistance to respiratory tract infections. Although there is experimental evidence that gases and vapors can be adsorbed onto carbonaceous particles, enhancing the toxicity of these particles when deposited in the lung, there is no evidence for an increased health risk from such interactions with DPM under ambient urban atmospheric conditions. Likewise, there is no experimental evidence in laboratory animals that the youth or preexisting emphysema of an exposed individual enhances the risk of exposure to diesel exhaust.

5.4. COMPARATIVE RESPONSIVENESS AMONG SPECIES TO THE PULMONARY EFFECTS OF DIESEL EXHAUST

There is some evidence indicating that species may differ in pulmonary responses to DE. Mauderly et al. (1993) compared the pulmonary histopathology of rats and mice after 18 mo of exposure to DE. There was less aggregation of macrophages. Diffuse septal thickening was noted in the mice, but there were few inflammatory cells, no focal fibrosis, little epithelial hyperplasia, and no epithelial metaplasia, as was observed in rats. Heinrich et al. (1986a) reported that wet lung weight of hamsters increased only 1.8-fold following chronic exposure to DE, compared with an increase of 3.4-fold in rats. Smaller increases in neutrophils, lactic acid dehydrogenase, collagen, and protein supported the conclusion of a lesser inflammatory response in Syrian hamsters. The histopathologic changes in the lungs of Chinese hamsters after 6 mo exposure to DE, on the other hand, was similar to that of rats (Pepelko and Peirano, 1983). Guinea pigs respond to chronic DE exposure with a well-defined epithelial proliferation, but it is based on an eosinophilic response in contrast to the neutrophil-based responses in other species. Epithelial hyperplasia and metaplasia were quite striking in the terminal and respiratory bronchioles of cats exposed for 27 mo to DE (Plopper et al., 1983). This study is of particular interest because the terminal airways of cats are more similar to those of humans than rodent species are. It should be noted, however, that exposure concentrations were very high (12 mg/m³) for most of the period. Lewis et al. (1989) exposed rats and Cynomolgous monkeys 8 hours per day, 5 days per week for 2 years to DE at a particle concentration of 2 mg/m³. Unfortunately, this exposure rate was sufficiently low that few effects were noted in either species other than focal accumulations of particles, primarily in the alveolar macrophages, interstitium,

and lymphoid tissue. To achieve chronic exposure conditions in monkeys would also require a considerably longer exposure period. It is apparent that species do vary in their pulmonary responses to DE exposure, despite the difficulty in making direct comparisons because of differences in exposure regimes, lifespans, and pulmonary anatomy. Most species do respond, however, suggesting that humans are likely to be susceptible to induction of pulmonary pathology during chronic exposure to DE.

5.5. DOSE-RATE AND PARTICULATE CAUSATIVE ISSUES

The purpose of animal toxicological experimentation is to identify the hazards and dose-response effects posed by a chemical substance or complex mixture and to extrapolate these effects to humans for subsequent health assessments. The cardinal principle in such a process is that the intensity and character of the toxic action are a function of the dose of the toxic agent(s) that reaches the critical site of action. The considerable body of evidence reviewed clearly denotes that major noncancerous health hazards may be presented to the lung following the inhalation of diesel exhaust. Based on pulmonary function and histopathological and histochemical effects, a determination can be made concerning what dose/exposure rates of diesel exhaust (expressed in terms of the DPM concentration) result in an injury to the lung and which appear to elicit no effect. The inhalation of poorly soluble particles, such as those found in diesel exhaust, increases the pulmonary particulate burden. When the dosing rate exceeds the ability of the pulmonary defense mechanisms to achieve a steady-state lung burden of particles, there is a slowing of clearance and the progressive retention of particles in the lung that can ultimately approach a complete cessation of lung clearance (Morrow, 1988). This phenomenon has practical significance both for the interpretation of experimental inhalation data and for the prevention of disease in humans exposed to airborne particles.

The data for exposure intensities that cause adverse pulmonary effects demonstrate that they are less than the exposure intensities reported to be necessary to induce lung tumors. Using the most widely studied laboratory animal species and the one reported to be the most sensitive to tumor induction, the laboratory rat, the no-adverse-effect exposure intensity for adverse pulmonary effects was $56 \text{ mg}\cdot\text{h}\cdot\text{m}^{-3}/\text{week}$ (Brightwell et al., 1986). The lowest-observed-effect level for adverse pulmonary effects (noncancer) in rats was $70 \text{ mg}\cdot\text{h}\cdot\text{m}^{-3}/\text{week}$ (Lewis et al., 1989), and for pulmonary tumors, $122.5 \text{ mg}\cdot\text{h}\cdot\text{m}^{-3}/\text{week}$ (Mauderly et al., 1987a). The results clearly show that noncancerous pulmonary effects are produced at lower exposure intensities than are pulmonary tumors. Such data support the position that inflammatory and proliferative changes in the lung may play a key role in the etiology of pulmonary tumors in exposed rats (Mauderly et al., 1990b).

Adults who have a preexisting condition that may predispose their lungs to increased particle retention (e.g., smoking or high particulate burdens from nondiesel sources), inflammation (e.g., repeated respiratory infections), epithelial proliferation (e.g., chronic bronchitis), and fibrosis (e.g., silica exposure) and infants and children, due to their developing pulmonary and immunologic systems, may have a greater susceptibility to the toxic actions of diesel exhaust. It should be noted that both the developing lung and a model of a preexisting disease state have been studied with regard to their effect on the lungs' response to diesel exhaust (Mauderly et al., 1990a, 1987b). Mauderly et al. (1987b) showed that diesel did not affect the developing lung more severely than the adult rat lung, and in fact, that clearance was faster in the younger lung. Mauderly et al. (1990a) compared the pulmonary response to inhalation of diesel exhaust in rats with elastase-induced emphysema with normal rats. They found that respiratory tract effects were not more severe in emphysematous rats and that the lung burden of particles was less in the compromised rat. These studies provide limited evidence that some factors that are often considered to result in a wider distribution of sensitivity among members of the population may not have this effect with diesel exposure. However, these studies have no counterpart in human studies and extrapolation to humans remains uncertain.

There is also the issue of whether the noncancerous health effects related to exposure to diesel exhaust are caused by the carbonaceous core of the particle or substances adsorbed onto the core, or both.

Current understanding suggests that much of the toxicity resulting from the inhalation of diesel exhaust relates to the carbonaceous core of the particles. Several studies on inhaled aerosols demonstrate that lung reactions characterized by an appearance of particle-laden AMs and their infiltration into the alveolar ducts, adjoining alveoli and tracheobronchial lymph nodes, hyperplasia of Type II cells, and the impairment of pulmonary clearance mechanisms are not limited to exposure to diesel particles. Such responses have also been observed in rats following the inhalation of coal dust (Lewis et al., 1989; Karagianes et al., 1986), titanium dioxide (Heinrich et al., 1995; Lee et al., 1985), carbon black (Nikula et al., 1995; Heinrich et al., 1995), titanium tetrachloride hydrolysis products (Lee et al., 1986), quartz (Klosterkotter and Buneman, 1961), volcanic ash (Wehner et al., 1986), amosite (Bolton et al., 1983), and manmade mineral fibers (Lee et al., 1988) among others. In more recent studies, animals have been exposed to carbon black that is similar to the carbon core of the diesel exhaust particle. Nikula et al. (1995) exposed rats for 24 mo to carbon black or diesel exhaust at target exposure concentrations of 2.5 and 6 mg/m³ (exposure rates of 200 or 520 mg·h·m⁻³/week). Both concentrations induced AM accumulation, epithelial proliferation, inflammation, and fibrosis. They observed essentially no difference in potency of nonneoplastic or in tumor responses based on a regression analysis.

Dungworth et al. (1994) reported moderate to severe inflammation characterized by multifocal bronchoalveolar hyperplasia, alveolar histiocytosis, and focal segmental fibrosis in rats exposed to carbon black for up to 20 mo at exposure rates of 510 to 540 mg·h·m⁻³/week. The observed lung pathology reflects notable dose-response relationships and usually evolves in a similar manner. With increasing dose, there is an increased accumulation and aggregation of particle-laden AMs, Type II cell hyperplasia, a foamy (degenerative) macrophage response, alveolar proteinosis, alveolar bronchiolization, cholesterol granulomas, and often squamous cell carcinomas and bronchioalveolar adenomas derived from metaplastic squamous cells in the areas of alveolar bronchiolization.

Heinrich et al. (1995) compared effects of diesel exposure in rats and mice with exposure to titanium dioxide or carbon black. Exposures to TiO₂ and carbon black were adjusted during the exposure to result in a similar lung burden for the three types of particles. At similar lung burdens in the rat, DPM, TiO₂, and carbon black had nearly identical effects on lung weights and on the incidence of lesions, both noncancer and cancer. Also, a similar effect on clearance of a labeled test aerosol was measured for the different particles. A comparison of the effect of DPM, TiO₂, and carbon black exposures in mice also showed a similar effect on lung weight, but noncancer effects were not reported and no significant increase in tumors was observed.

These experiments provide strong support for the idea that diesel exhaust toxicity results from a mechanism that is analogous to that of other relatively inert particles in the lung. This qualitative similarity exists along with some apparent quantitative differences in the potency of various particles for producing effects on the lung or on particle clearance.

Particle size, volume, surface area, and composition may be the critical elements in the overload phenomenon following exposure to particles, which could explain those quantitative differences. The overloaded AMs secrete a variety of cytokines, oxidants, and proteolytic enzymes that are responsible for inducing particle aggregation and damaging adjacent epithelial tissue (Oberdörster and Yu, 1991). For a more detailed discussion of mechanism, see Chapter 10.

The principal noncancerous health hazard to humans posed by exposure to diesel exhaust is a structural or functional injury to the lung based on the laboratory animal data. Such effects are demonstrable at dose rates or cumulative doses of DPM lower than those reported to be necessary to induce lung tumors. Current knowledge indicates that the carbonaceous core of diesel particles is the major causative factor in the injury to the lung and that other factors such as the cytotoxicity of adsorbed substances on the particles also may play a role. The lung injury appears to be mediated through effects on pulmonary AMs. Because noncancerous pulmonary effects occur at lower doses than tumor induction does in the rat, and because these effects may be cofactors in the etiology of diesel exhaust-induced tumors, noncancerous pulmonary effects must be considered in the total evaluation of diesel exhaust, notably the particulate component.

5.6. SUMMARY AND DISCUSSION

5.6.1. Effects of Diesel Exhaust on Humans

The most readily identified acute noncancer health effect of diesel exhaust on humans is its ability to elicit subjective complaints of eye, throat, and bronchial irritation and neurophysiological symptoms such as headache, lightheadedness, nausea, vomiting, and numbness and tingling of the extremities. Studies of the perception and offensiveness of the odor of diesel exhaust and a human volunteer study in an exposure chamber have demonstrated that the time of onset of the human subjective symptoms is inversely related to increasing concentrations of diesel exhaust and the severity is directly related to increasing concentrations of diesel exhaust. In one study in which a diesel engine was operated under varying load conditions, a dilution factor of 140 to 475 was needed to reduce the exhaust level to an odor-detection threshold level.

A public health issue is whether short-term exposure to diesel exhaust might result in an acute decrement in ventilatory function and whether the frequent repetition of such acute respiratory effects could result in chronic lung function impairment. One convenient means of studying acute decrements in ventilatory function is to monitor differences in pulmonary function in occupationally exposed workers at the beginning and end of a workshift. In studies of underground miners, bus garage workers, dock workers, and locomotive repairmen, increases in respiratory symptoms (cough, phlegm, and dyspnea) and decreases in lung function (FVC, FEV₁, PEFR, and FEF₂₅₋₇₅) over the course of a workshift were generally found to be minimal and not statistically significant. In a study of acute respiratory responses in diesel bus garage workers, there was an increased reporting of cough, labored breathing, chest tightness, and wheezing, but no reductions in pulmonary function were associated with exposure to diesel exhaust. Pulmonary function was affected in stevedores over a workshift exposure to diesel exhaust but normalized after a few days without exposure to diesel exhaust fumes. In a third study, there was a trend toward greater ventilatory function changes during a workshift among coal miners, but the decrements were similar in miners exposed and not exposed to diesel exhaust.

Smokers appeared to demonstrate larger workshift respiratory function decrements and increased incidents of respiratory symptoms. Acute sensory and respiratory symptoms were earlier and more sensitive indicators of potential health risks from diesel exposure than were decrements in pulmonary function. Studies on the acute health effects of exposure to diesel exhaust in humans, experimental and epidemiologic, have failed to demonstrate a consistent pattern of adverse effects on respiratory morbidity; the majority of studies offer, at best, equivocal evidence for an exposure-response relationship. The environmental contaminants have frequently been below permissible workplace exposure limits; in those few cases where health effects have been reported, the authors have failed to identify conclusively the individual or collective causative agents in the diesel exhaust.

Chronic effects of diesel exhaust exposure have been evaluated in epidemiologic studies of occupationally exposed workers (metal and nonmetal miners, railroad yard workers, stevedores, and bus garage mechanics). Most of the epidemiologic data indicate an absence of an excess risk of chronic respiratory disease associated with exposure to diesel exhaust. In a few studies, a higher prevalence of respiratory symptoms, primarily cough, phlegm, or chronic bronchitis, was observed among the exposed. These increased symptoms, however, were usually not accompanied by significant changes in pulmonary function. Reductions in FEV₁ and FVC and, to a lesser extent, FEF₅₀ and FEF₇₅, also have been reported. Two studies detected statistically significant decrements in baseline pulmonary function consistent with obstructive airway disease. One study of stevedores had a limited sample size of 17 exposed and 11 controls. The second study in coal miners showed that both underground and surface workers at diesel-use mines had somewhat lower pulmonary performance than their matched controls. The proportion of workers in or at diesel-use mines, however, showed equivalent evidence of obstructive airway disease and for this reason the authors of the second paper felt that factors other than diesel exposure might have been responsible. A doubling of minor restrictive airway disease was also observed in workers in or at diesel-use mines. These two studies, coupled with other reported nonsignificant trends in respiratory flow-volume measurements, suggest that exposure to diesel exhaust may impair pulmonary function among occupational populations. Epidemiologic studies of the effects of diesel exhaust on organ systems other than the pulmonary system are scant. Whereas a preliminary study of the association of cardiovascular mortality and exposure to diesel exhaust found a fourfold higher risk ratio, a more comprehensive epidemiologic study by the same investigators found no significant difference between the observed and expected number of deaths caused by cardiovascular disease.

Caution is warranted in the interpretation of results from the epidemiologic studies that have addressed noncarcinogenic health effects from exposure to diesel exhaust. These investigations suffer from myriad methodological problems, including (1) incomplete information on the extent of exposure to diesel exhaust, necessitating in some studies estimations of exposures from job titles and resultant misclassification; (2) the presence of confounding variables such as smoking or occupational exposures to other toxic substances (e.g., mine dusts); and (3) the short duration and low intensity of exposure. These limitations restrict drawing definitive conclusions as to the cause of any noncarcinogenic diesel exhaust effect, observed or reported.

5.6.2. Effects of Diesel Exhaust on Laboratory Animals

Laboratory animal studies of the toxic effects of diesel exhaust have involved acute, sub-chronic, and chronic exposure regimens. In acute exposure studies, toxic effects appear to have been associated primarily with high concentrations of carbon monoxide, nitrogen dioxide, and

aliphatic aldehydes. In short- and long-term studies, toxic effects have been associated with exposure to the complex exhaust mixture. Effects of diesel exhaust in various animal species are summarized in Tables 5-2 to 5-14. In short-term studies, health effects are not readily apparent, and when found, are mild and result from concentrations of about 6 mg/m³ DPM and durations of exposure approximating 20 h/day. There is ample evidence, however, that short-term exposures at lower levels of diesel exhaust affect the lung, as indicated by an accumulation of DPM, evidence of inflammatory response, AM aggregation and accumulation near the terminal bronchioles, Type II cell proliferation, and the thickening of alveolar walls adjacent to AM aggregation. Little evidence exists, however, from short-term studies that exposure to diesel exhaust impairs lung function. Chronic exposures cause lung pathology that results in altered pulmonary function and increased DPM retention in the lung. Exposures to diesel exhaust have also been associated with increased susceptibility to respiratory tract infection, neurological or behavioral changes, an increase in banded neutrophils, and morphological alterations in the liver.

5.6.2.1. *Effects on Survival and Growth*

The data presented in Table 5-3 show limited effects on survival in mice and rats and some evidence of reduced body weight in rats following chronic exposures to concentrations of 1.5 mg/m³ DPM or higher and exposure durations of 16 to 20 h/day, 5 days/week for 104 to 130 weeks. Increased lung weights and lung to body weight ratios in rats, mice, and hamsters; an increased heart to body weight ratio in rats; and decreased lung and kidney weights in cats have been reported following chronic exposure to diesel exhaust. No evidence was found of an effect of diesel exhaust on other body organs (Table 5-4). The lowest-observed-effect level in rats approximated 1 to 2 mg/m³ DPM for 7 h/day, 5 days/week for 104 weeks.

5.6.2.2. *Effects on Pulmonary Function*

Pulmonary function impairment has been reported in rats, hamsters, cats, and monkeys exposed to diesel exhaust and included lung mechanical properties (compliance and resistance), diffusing capacity, lung volumes, and ventilatory performance (Table 5-5). The effects generally appeared only after prolonged exposures. The lowest exposure levels (expressed in terms of DPM concentrations) that resulted in impairment of pulmonary function occurred at 2 mg/m³ in cynomolgus monkeys (the only level tested), 1.5 and 3.5 mg/m³ in rats, 4.24 and 6 mg/m³ in hamsters, and 11.7 mg/m³ in cats. Exposures in monkeys, cats, and rats (3.5 mg/m³) were for 7 to 8 h/day, 5 days/week for 104 to 130 weeks. While this duration is considered to constitute a lifetime study in rodents, it is a small part of the lifetime of a monkey or cat, so these effect levels are essentially comparing chronic rodent studies with a subchronic monkey study. Exposures in hamsters and rats (1.5 mg/m³) varied in hours per day (8 to 20) and weeks of exposure (26 to

130). In all species but the monkey, the testing results were consistent with restrictive lung disease; alteration in expiratory flow rates indicated that 1.5 mg/m³ DPM was a LOAEL for a chronic exposure (Gross, 1981). Monkeys demonstrated evidence of obstructive airway disease. The nature of the pulmonary impairment is dependent on the dose of toxicants delivered to and retained in the lung, the site of deposition and effective clearance or repair, and the anatomy and physiology of the affected species; these variables appear to be factors in the disparity of the airway disease in monkey versus the other species tested.

5.6.2.3. *Histopathological and Histochemical Effects*

Histological studies have demonstrated that chronic exposure to diesel exhaust can result in effects on respiratory tract tissue (Table 5-6). Typical findings include alveolar histiocytosis, AM aggregation, tissue inflammation, increase in PMNs, hyperplasia of bronchiolar and alveolar Type II cells, thickened alveolar septa, edema, fibrosis, and emphysema. Lesions in the trachea and bronchi were observed in some studies. Associated with these histopathological findings were various histochemical changes in the lung, including increases in lung DNA, total protein, alkaline and acid phosphatase, glucose-6-phosphate dehydrogenase; increased synthesis of collagen; and release of inflammatory mediators such as leukotriene LTB and prostaglandin PGF_{2α}. Although the overall laboratory evidence is that prolonged exposure to DPM results in histopathological and histochemical changes in the lungs of exposed animals, some studies have also demonstrated that there may be a threshold of exposure to DPM below which pathologic changes do not occur. These no-observed-adverse-effect levels for histopathological effects were reported to be 2 mg/m³ for cynomolgus monkeys (the only concentration tested), 0.11 to 0.35 mg/m³ for rats, and 0.25 mg/m³ DPM for guinea pigs exposed for 7 to 20 h/day, 5 to 5.5 days/week for 104 to 130 weeks.

5.6.2.4. *Effects on Defense Mechanisms*

The pathological effects of DPM appear to be strongly dependent on the relative rates of pulmonary deposition and clearance (Table 5-7). Clearance of particles from the alveolar region of the lungs is a multiphasic process involving phagocytosis by AMs. Chronic exposure to DPM concentrations of about 1 mg/m³ or above, under varying exposure durations, causes pulmonary clearance to be reduced with concomitant focal aggregations of particle-laden AMs, particularly in the peribronchiolar and alveolar regions, as well as in the hilar and mediastinal lymph nodes. The exposure concentration at which focal aggregates of particle-laden AMs occur may vary from species to species, depending on rate of uptake and pulmonary deposition, pulmonary clearance rates, the relative size of the AM population per unit of lung tissue, the rate of recruitment of AMs and leukocytes, and the relative efficiencies for removal of particles by the mucociliary and

lymphatic transport system. The principal mechanism of reduced particle clearance appears to be an effect on pulmonary AMs. Impairment of particle clearance seems to be nonspecific and applies primarily to dusts that are persistently retained in the lungs. Lung dust levels of approximately 0.1 to 1 mg/g lung tissue appear to produce this effect in the Fischer 344 rat (HEI, 1995). Morrow (1988) suggested that the inability of particle-laden AMs to translocate to the mucociliary escalator is correlated to an average composite particle volume per AM in the lung. When this particle volume exceeds approximately $60 \mu\text{m}^3$ per AM in the Fischer 344 rat, impairment of clearance appears to be initiated. When the particulate volume exceeds approximately $600 \mu\text{m}^3$ per cell, evidence suggests that AM-mediated particulate clearance virtually ceases and agglomerated particle-laden macrophages remain in the alveolar region and increasingly nonphagocytized dust particles translocate to the pulmonary interstitium. Data for other laboratory animal species and humans are, unfortunately, limited.

Several laboratory animal studies have indicated that exposure to DPM can reduce an animal's resistance to respiratory infections. This effect, which can occur even after only 2 or 6 h of exposure to diesel exhaust containing 5 to 8 mg/m^3 DPM, does not appear to be caused by direct impairment of the lymphoid or splenic immune systems; however, in one study of influenza virus infection, interferon levels and hemagglutinin antibody levels were adversely affected in the exposed mice. Studies on the effects of exposure to diesel exhaust or DPM on the immune system of laboratory animals have produced equivocal results (Table 5-8).

5.6.2.5. *Neurological and Behavioral Effects*

Behavioral effects have been observed in rats exposed to diesel exhaust from birth to 28 days of age (Table 5-13). Exposure caused a decreased level of spontaneous locomotor activity and a detrimental effect on learning in adulthood. In agreement with the behavioral changes was physiological evidence for delayed neuronal maturation. Exposures were to 6 mg/m^3 DPM for 8 h/day, 7 days/week from birth to about 7, 14, 21, or 28 days of age.

5.6.2.6. *Other Noncancerous Effects*

Essentially no effects (based on the weight of evidence of a number of studies) were noted for reproductive and teratogenic effects in mice, rats, rabbits, and monkeys; clinical chemistry and hematology in the rat, cat, hamster, and monkeys; and enzyme induction in the rat and mouse (Tables 5-10 through 5-12 and 5-14).

5.6.3. Comparison of Filtered and Unfiltered Diesel Exhaust

The comparison of the toxic responses in laboratory animals exposed to whole diesel exhaust or filtered exhaust containing no particles demonstrates across laboratories that diesel

particles are the principal etiologic agent of noncancerous health effects in laboratory animals exposed to diesel exhaust (Table 5-15). Whether the particles act additively or synergistically with the gases cannot be determined from the designs of the studies. Under equivalent exposure regimens, hamsters have lower levels of retained DPM in their lungs than rats and mice do and consequently less pulmonary function impairment and pulmonary pathology. These differences may result from a lower intake rate of DPM, lower deposition rate and/or more rapid clearance rate, or lung tissue that is less susceptible to the cytotoxicity of DPM. Observations of a decreased respiration in hamsters when exposed by inhalation favor lower intake and deposition rates.

5.6.4. Interactive Effects of Diesel Exhaust

There is no direct evidence that diesel exhaust interacts with other substances in an exposure environment, other than an impaired resistance to respiratory tract infections. Young animals were not more susceptible. In several ways, animals with laboratory-induced emphysema were more resistant. There is experimental evidence that both inorganic and organic compounds can be adsorbed onto carbonaceous particles. When such substances become affiliated with particles, these substances can be carried deeper into the lungs where they might have a more direct and potent effect on epithelial cells or on AM ingesting the particles. Few specific studies to test interactive effects of diesel exhaust with atmospheric contaminants, other than coal dust, have been conducted. Coal dust and DPM had an additive effect only.

5.6.5. Summary

The principal noncancerous health hazard to humans from exposure to diesel exhaust is a structural, functional, or biochemical injury to the lung. Current knowledge indicates that the carbonaceous core of the diesel particle is the prime causative agent of lung injury. The lung injury appears to be mediated by a progressive impairment of AMs. Because noncancerous pulmonary effects occur at lower doses than those inducing tumors in rats and appear to be cofactors in the etiology of diesel exhaust-induced tumors in rats, noncancerous pulmonary effects are relevant factors in the development of risk assessments.

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